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ASPECTS OF THE ACTIVITY OF THE RENIN - ANGIOTENSIN SYSTEM IN HYPERTENSION

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ASPECTS OF THE ACTIVITY OF THE RENIN-ANGIOTENSIN SYSTEM IN HYPERTENSION

PROEFSCHRIFT

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Aan mijn ouders,

Thea en Mijke.

CONTENTS

INTRODUCTION

Since the middle of the sixties the Division of Endocrinology of the Department of Medicine of the Nijmegen University Hospital has paid attention to aspects of the activity of the renin - angiotensin system and to the production of mineralocorticoids in hypertensive syndromes, in order to detect endocrine causes of hypertension.

The dispute on the significance of the renin state of the hypertensive patient has become increasingly lively in recent years. Extreme points of view have been taken by Laragh and coworkers in New York City, who claim an almost prophetic role of the activity of the renin - angiotensin system in the natural course of the hypertensive process, and by the Glasgow group, headed by Lever, who look at the renin activity in the peripheral blood as a remote marker of adjustments of the kidneys to the circulatory derangements in hypertension. Neither of these extreme views on the significance of the activity of the renin-angiotensin system seem to account for a number of phenomena observed untill the present in this as well as in other laboratories.

In 1969 Driessen of our group (doctoral thesis, Nijmegen), paid attention to the blunted response of the renin - angiotensin system in benign essential hypertension to stimuli such as sodium restriction and upright posture. In those days renin activity was measured by the well known Boucher's bioassay. Today, renin activity is assessed by radioimmunoassay of generated angiotensin I. Because of a still not definitively closed discussion about the correspondence between the results of the bioassay and the radioimmunoassay a study was made, which is reported in Chapter I, dealing with the comparison of both methods. This study will be published in Clinica Chimica Acta (in press).

In Chapters II and III attention is paid to methods to

detect 'low renin' and 'normal renin' hypertensive patients on an out-patient base in a meaningful way. In recent literature a number of differing methods have been used to characterize the renin state of normotensive and hypertensive individuals. Part of the conflicting data and concepts based on them in the renin field might be explained by important differences in the methodology of the detection of the renin state. Part of this study was published in Clinical Science and Molecular Medicine, 1975, 48, 91 - 96.

In Chapter V a study is presented aiming to relate the renin state of hypertensive patients to the effect of differing modes of drug treatment. This study is part of a collaborative effort of the Division of Endocrinology and the Out-patient Division of the Department of Internal Medicine. In two groups of patients, one composed of normoreninemics, the other of hyporeninemics, effects of long-term treatment with the saluretic drug chlorthalidone, with the anti-aldosterone drug spironolactone and with the β-blocker propranolol, a number of variables have been, and will be studied. Chapter V discloses results of the study in a representative sample of normoreninemic hypertensive patients. The effects of these varying drug schedules on the blood pressure were of prime importance. Because of the limitations of casual auscultatory blood pressure measurement in this type of study automated blood pressure recording was practized instead. In Chapter IV a comparative study of blood pressure recording by classical manometry and by the automated device used has been reported.

Chapter I

THE RELIABILITY OF THE MEASUREMENT OF PLASMA RENIN ACTIVITY BY RADIOIMMUNOASSAY.

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SUMMARY

A radioimmunoassay of angiotensin I has been applied to the measurement of plasma renin activity. Angiotensin I was generated in plasma samples by 3 h incubation at 37° and pH 5.6 after addition of EDTA and Dowex. The generated amount of angiotensin I was measured by radioimmunoassay in the eluate of the Dowex column. With this method a negligible amount of angiotensin I was measured after incubation at 4° C (0.8 ng/ml per 3 h). Eluate of blank plasma had no measurable effect on the standard curve. The mean recovery of angiotensin I was 87 %. The limit of detection of the assay was 0.5 ng/ml per 3 h. The results obtained using different antisera were equal. A marked variation was found in immunological properties of the different standard preparations of angiotensin I tested. The mean value of angiotensin I generation per Goldblatt Unit (G.U.) renin was $3.9 \cdot 10^{4}$ ng/h. In normotensive control subjects, the plasma renin concentration, while on unrestricted diet and after 3 h ambulation, was on average $0.39 \cdot 10^{-4}$ G.U./ml, range $0.12 \cdot 10^{-4} - 0.91 \cdot 10^{-4}$.

With the use of the same plasma extracts for radioimmunoassay and bioassay, a perfect correlation was found between the plasma renin activities measured with both assays. The differences found between the results of both assays could be fully explained by the different biological activities of the standards used (Angiotensin I, Schwarz Mann, and Angiotensin II, Ciba-Geigy).

With a direct radioimmunoassay, angiotensin I was generated in plasma by 3 h incubation at 37° C and pH 5.6 after addition of phenylmethanesulfonyl fluoride, 8-hydroxyquinoline and 2.3-dimercaptopropanol (dimercaprol). The generated amount of angiotensin I was measured by the above mentioned radioimmunoassay. A fair correlation was found

between the generated amounts of angiotensin I measured in the Dowex eluate and those found in the incubated plasma. Especially in the lowest range, lower values were obtained by the latter assay. However, the generated amounts of angiotensin I measured in non-incubated plasma samples (3 h at 4° C) was on average 6.4 ng/ml per 3 h and accounted for 7 - 48 % of the amounts found after incubation at 37° C.

Various laboratories discontinued the use of the bioassay method for the measurement of plasma renin activity (PRA) and developed a radioimmunoassay system. There are obvious reasons for such developments because the bioassay technique, although reliable, is a relatively insensitive, difficult and laborious procedure. Unfortunately, the results obtained by radioimmunoassay have been described to deviate widely, some investigators (1, 2) reporting lower, most however (3 - 7), higher values than obtained by bioassay. The variability in incubation conditions for generating angiotensin and the use of different angiotensin standards have been reported to be the explanation for these differences (6, 8). However, the generation of material cross-reacting in the immunoassay, the presence of biologically inactive though immunologically active fragments in the immunoassay are still considered to be more or less responsible for the discrepancies found between both assays (4, 7 - 10).

In this report the results of measurement of plasma renin activity by bioassay were compared with those obtained by radioimmunoassay. Angiotensin I was generated under identical conditions in both assays using Dowex extraction. The radioimmunoassay after purification was compared with a simplified radioimmunoassay procedure without extraction of the incubation product. Sensitivity and accuracy of the radioimmunoassay were analyzed and attention was paid to calibration with the use of the international standard of renin.

Albumin, bovine 96 - 99 % (Sigma).

Ammonium acetate (Merck), 0.2 N solution in sterile distilled water, acidified to pH 6 with glacial acetic acid.

Ammonium hydroxide (Merck), 0.2 N solution in sterile distilled water.

Angiotensin I standards:

Asp^1 - Ile^5 Angiotensin I (Schwarz Mann, Orangeburg, New York, USA), batch November 1972 and May 1974.

Asp^1 - Ile^5 synthetic Angiotensin I 71/328 (Medical Research Council, Divison of Biological Standards, National Institute for Medical Research, Hampstead Laboratories, Holly Hill, London, England).

Human synthetic angiotensin I (Beckman Instruments Inc., Geneva, Switzerland).

Ile^5 angiotensin I (Sorin, Inco, Amerongen, The Netherlands). All standards diluted with Tris buffer and stored in 50 µl aliquots, containing 2500 ng, at - 20° C. This solution keeps for at least 6 months.

Angiotensin II standard:

Asp^1 -β amide-Val^5-Angiotensin II (Hypertensin, Ciba-Geigy).

Antiserum A and B, raised in rabbits against angiotensin I in 1972 and 1973 respectively (generously supplied by Dr. H.J.G. Hollemans, Department of Endocrinology, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands). The antisera were stored, diluted 1 : 1000 in Tris buffer, in 0.2 ml samples at - 20° C.

Bovine serum albumin, BSA (Behringwerke AG), 5 % solution in sterile distilled water.

Butan-1-ol (Merck).

DASP, sheep anti-rabbit γ-globulin immunosorbent, vials

of 5.5 ml (Organon, Oss, The Netherlands), each vial diluted with Tris buffer to 25 ml.

Dibenzyline, Dibenamine (phenoxybenzamine) (Smith, Kline and French Labs Ltd), 0.05 % solution in sterile distilled water.

Diethylamine (Merck), fraction-distilled twice at 5.5° C, 0.1 N solution in sterile distilled water,

2.3 Dimercapto - 1 - propanol (Fluka AG).

Disodiumhydrogen phosphate . 12 H_2O. (Merck) pure crystalline.

Disodium EDTA, Titriplex III (Merck), 10 % solution in sterile distilled water.

Dowex 50W - x2, 100 - 200 mesh (Baker's Analyzed Reagent). After washing the Dowex with distilled water (2000 ml per 500 g) the Dowex was poured into a column with a glasswool filter. Subsequently 2 x 1000 ml 4 N NaOH was poured onto the Dowex. After washing with 2 x 500 ml distilled water the column was washed first with 1000 ml 2 N HCl and distilled water to pH 7.0 and secondly with 4000 ml 0.2 N ammonium acetate to pH 6.0. The Dowex (NH_4^+) prepared in this way was stored at 4° C until needed.

Ethanol 100 % (Merck), 80 % solution in sterile distilled water.

Ether anaestheticus (Kon. Ned. Gist- en Spiritusfabrieken).

Glacial acetic acid 99 % (Merck), 10 % solution in sterile distilled water.

HCl (Merck), 0.01, 1, 2 and 4 N solutions in sterile distilled water.

8-Hydroxyquinoline sulfate (Fluka AG).

(^{125}I)-Angiotensin I, CEA - Sorin (Ire, Moll, Belgium) stored at - 20° C.

Lysozume, (Boehringer).

Merthiolate, Thiomersal (BDH).

NaCl (Merck).

NaOH (Merck), 4 N solution in sterile distilled water.

Neomycin sulfate (Lundbeck).

Pentolinium tartrate 0.5 %, Ansolysen (May and Baker Ltd).

Phenylmercury acetate (BDH).

Phenylmethanesulfonyl fluoride, PMSF (Merck).

Phosphate buffer, pH 7.6, containing per 1000 ml sterile distilled water, 5.74 g disodiumhydrogen phosphate . 12 H_2O, 0.42 g potassiumhydrogen phosphate and 0.1 g merthiolate.

Potassiumhydrogen phosphate (Merck).

PMSF solution, containing per 10 ml sterile distilled water, 1 g neomycin sulfate, 500 mg PMSF, 500 mg 8-hydroxy-quinoline sulfate and 0.32 µmol dimercaprol.

Standard renin, 0.1 Goldblatt Unit per ampoule (M.R.C., Hampstead Laboratories, Holly Hill, London, England). The content of one ampoule was dissolved in 2 ml Tris buffer and 10 or 20 µl of this solution, containing 1/2000 and 1/1000 G.U. respectively, was added to plasma samples.

Sterile distilled water.

Thymol blue indicator (BDH).

Tris buffer, containing per 1000 ml sterile distilled water: 12.1 g Tris, 3.0 g BSA, 2.0 g neomycin sulfate, 0.034 g phenylmercury acetate, 3.0 g lysozyme. This solution was acidified to pH 7.5 with glacial acetic acid.

Trizma base (Sigma).

Urethane ethylcarbamate (BDH), 50 % solution in sterile distilled water.

METHODS

Blood was collected in ice-cooled containers. Disodium EDTA, 0.1 ml of 10 % solution per 10 ml blood, was used as

an anticoagulant and as a blocker of the converting enzyme and the angiotensinase activity. The blood was centrifuged for 15 minutes, 3000 rpm at 4° C and the plasma was stored at - 20° C until assay.

Bioassay of the plasma renin activity.

Angiotensin I generation and purification.

Plasma was thawed avoiding a rise in temperature above 4° C. To 10 ml plasma 4 ml of the Dowex suspension was added and the mixture was acidified to pH 5.6 with 1 N HCl. Incubation was carried out for 3 hours at 37° C while gently shaking. After incubation the mixture was poured into a column containing 1 ml Dowex suspension. The column was washed with 30 ml 0.2 N ammonium acetate, 30 ml 10 % acetic acid and 30 ml distilled water subsequently. The angiotensin I, generated during incubation, was eluted from the column with 15 ml 0.1 N diethylamine and 20 ml 0.2 N ammonium hydroxide. To the eluate, acidified with 0.25 ml glacial acetic acid, 10 ml 80 % ethanol was added. The solution was concentrated to 2 ml under reduced pressure by flask-evaporation and subsequently heated in a boiling water bath for 15 minutes. After cooling to room temperature the solution was acidified to pH 5.0 with 4 N HCl, using thymol blue as an indicator, and saturated with sodium chloride. Extraction of the angiotensin was performed with 25, 10 and 8 ml butanol and the angiotensin I was reextracted with 3 x 15 and 2 x 5 ml 0.01 N HCl. After addition of 4 ml 80 % ethanol, the aqueous alcoholic solution was, under reduced pressure, flask-evaporated to dryness. The residue was dissolved in 2 ml 5 % bovine serum albumin just before bioassay.

In some experiments the angiotensin I was assayed by

radioimmunoassay of the final bioassay extract. In these cases the residue was dissolved in Tris buffer.

Angiotensin I bioassay.

The angiotensin I bioassay was performed in two nephrectomized male Wistar rats. The nephrectomy was carried out under ether anaesthesia about 18 hours before bioassay. Depending on the weight of the rats (190 - 270 g) an intra-abdominal injection of 0.39 - 0.46 ml 50 % urethane was given as an anaesthetic. A tracheotomy was performed to prevent airway obstruction. Two cannulae were placed in the right jugular vein, one for the administration of the standard angiotensin II and one for the injection of the final bioassay extract. The blood pressure was measured by a Statham pressure transducer connected with an indwelling catheter in the left carotid artery. A weight related dose of 0.5 % pentolinium tartrate (0.4 - 1.2 ml) and 0.05 % dibenzyline (0.80 - 1.90 ml) was given subcutaneously, resulting in a final blood pressure of 40 - 80 mmHg. After about one hour the pressor effect of a volume of the final extract BSA solution was compared to the pressor effect of known amounts of the angiotensin II standard BSA 5 % solution in both rats. The final extract of an unknown plasma was assayed twice in each rat, using single and double doses of the extract.

The bioassay of the PRA described here is essentially the procedure of Boucher et al. (11) modified in this laboratory (12). The coefficient of variation, determined by analysing plasma pools repeatedly, was 9, 10 and 12 % for pools containing 5, 12 and 32 ng angiotensin/ml per 3 h respectively. The recovery of the bioassay was 89 $\pm$ 11 %.

Radioimmunoassay of the plasma renin activity.

Procedure A.

Angiotensin I generation and purification.

The volume of the plasma analyzed was 2.5 ml. The incubation, washing and elution procedures were carried out as described for the bioassay, using the following volumes of reagents. One ml of the Dowex suspension was added to the plasma. The column, containing 1 ml Dowex suspension, was washed with 30 ml 0.2 N ammonium acetate, 30 ml 10 % acetic acid and 30 ml distilled water. The angiotensin I was eluted with 10 ml 0.1 N diethylamine and 15 ml 0.2 N ammonium hydroxide. The eluate was acidified with 0.2 ml glacial acetic acid. According to the amount of angiotensin I expected to be present in the eluate, the eluate was used either undiluted or after dilution with Tris buffer or after concentration by evaporation of an appriopriate amount of solvent on a water-bath at 60° C.

Angiotensin I radioimmunoassay.

The incubation mixture for the radioimmunoassay was prepared as follows:

50 µl antiserum (final dilution of antiserum A 1 : 240 000 and antiserum B 1 : 1 100 000).

50 µl ^{125}I-labeled angiotensin I (about 3000 cpm, containing 1.5 - 8 pg angiotensin I).

20 µl or 40 µl of the unknown sample or

20 µl Tris buffer containing standard angiotensin I

900 µl Tris buffer.

The incubation was performed for 20 hours at 4° C. After incubation 0.5 ml DASP-suspension was added to separate the antibody-bound and free hormone. The mixture was rotated

for 2.5 hours at 4° C. After centrifugation the supernatant was aspirated and the residue washed twice with 2 ml phosphate buffer. After the final centrifugation, the precipitate, containing the bound hormone, was counted in a Wallac sample counter (GTL 300 - 500).

The PRA of the plasma samples was calculated according to the formula: $x = a/b \cdot (c \cdot 10)$ in which x is the PRA (ng/ml per 3 h), a is the amount of angiotensin I present in the DASP precipitate (pg), b is the amount of Dowex eluate added to the radioimmunoassay incubation mixture (µl) and c is the dilution or concentration factor of the Dowex eluate. In the routine assay procedure the antiserum B was used with the angiotensin I standard from Schwarz Mann.

Procedure B.

Angiotensin I generation and radioimmunoassay.

0.1 ml PMSF solution was added to 2.5 ml plasma. The plasma was adjusted to pH 5.6 with 1 N HCl. Two 1 ml samples of the plasma were incubated for 3 hours at 4 and 37° C, respectively. To this point the procedure described by Freedlender et al. (13) was followed. The angiotensin I generated in the incubated samples was radioimmunoassayed as described for Procedure A.

Standard statistical tests were used to detect differences between two groups and to calculate correlation coefficients. The results are expressed as means ± standard deviation.

RESULTS

Unless stated, the results presented were obtained using radioimmunoassay procedure A as described in the methods section.

Standard curve.

Routinely, the standard curve of angiotensin I was set up in triplicate. Fig. 1 shows the mean and standard deviation of triplicates from 6 standard curves obtained on different days within a period of two months. The standard deviation for antibody alone (B_o) was 0.9 % and after addition of 10, 25,50 and 100 pg angiotensin I this value ranged from 0.9 to 1.1 %. After addition of 150 and 250 pg angiotensin I the standard deviation was 0.4 %. Only that part of the curve between 10 and 200 pg angiotensin I was used in practice.

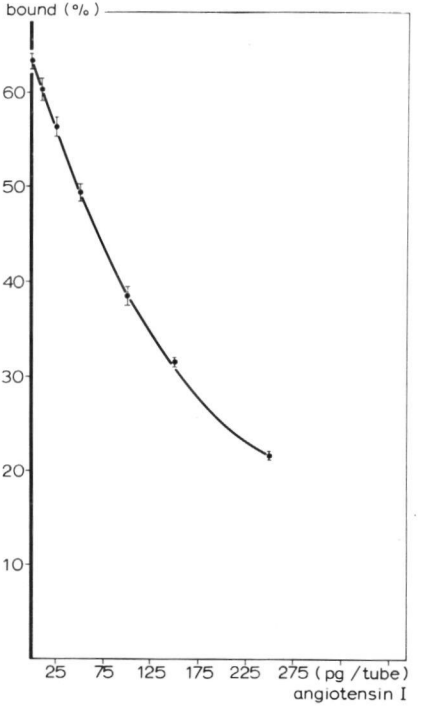

Fig. 1. MEAN AND STANDARD DEVIATION OF 6 STANDARD CURVES. EACH STANDARD CURVE WAS ASSAYED IN TRIPLICATE WITH THE RADIOIMMUNOASSAY PROCEDURE A.

Blank.

Dowex eluates (20 and 40 µl) of plasma samples of bilaterally nephrectomized patients added to the entire range of the standard curve did not alter the binding of the labelled hormone to the antibody. Therefore the blank of the method appeared to be zero.

Precision and sensitivity of the radioimmunoassay.

The precision obtained in the assay of plasma samples was studied by carrying out duplicate assays of plasma eluates containing different ranges of angiotensin I concentrations (Table 1).

Table 1. PRECISION AND SENSITIVITY OF DUPLICATE ASSAYS OF PRA IN PLASMA SAMPLES MEASURED BY RADIOIMMUNOASSAY (PROCEDURE A).

RANGE OF PLASMA RENIN ACTIVITY VALUES IN THE PLASMA SAMPLES ng/ml.3h	NUMBER OF DUPLICATES n	COEFFICIENT OF VARIATION %	PRECISION ng/ml.3h	SENSITIVITY ($p < 0.05$) ng/ml 3h
0 - 5	21	14 6	0.4	0.5
5 - 10	14	12.3	0.9	1.3
10 - 30	32	12.4	2.5	3 6
30 - 60	28	5.9	2 5	3 7
60 - 90	21	5.1	3.6	5 3

The precision, S, in ng/ml per 3 h was calculated according to the formula

$$S = \frac{1}{2n}\sqrt{\sum_{i=1}^{n} (x_{i1} - x_{i2})^2}$$

in which x_{i1} and x_{i2} are the values of the i-th duplicate and n is the number of the duplicate pairs. From this

precision, the sensitivity of the method in a duplicate assay was calculated according to the formula

$$\text{sensitivity (ng/ml per 3 h)} = t \times S/\sqrt{2}$$

in which t is the critical value at the 5 % level of the Student-t test for n degrees of freedom. The sensitivity is the smallest duplicate mean value differing significantly ($p < 0.05$) from zero.

From the results presented in table 1 the sensitivity appeared to be 0.5 ng/ml per 3 h.

The limit of detection has also been predicted by analysing the variance of the blank of the standard curve, assuming equality of blank and sample variances, and using a one-sided t-test at $p < 0.05$ (14). In this way the predicted limit of detection was calculated to be 5 pg per tube. Assuming this amount to be present in 40 µl of the concentrated (5 times) eluate, this results in a sensitivity of 0.25 ng/ml per 3 h. Obviously the actual sensitivity is lower than this theoretical value.

Between assay replicate variation.

Three pooled plasma samples with different PRA values were analyzed repeatedly on separate days. From the results presented in Table 2 it appears that the coefficient of variation was about 13 % and did not differ substantially between the different plasma pools.

Parallelism.

Parallelism of the immunological response of generated angiotensin I and standard angiotensin I was evaluated by comparing the angiotensin I concentrations (pg/µl) measured

Table 2. BETWEEN ASSAY REPLICATE VARIATION OF THE RADIO-IMMUNOASSAY (PROCEDURE A).

PLASMA POOL	NUMBER OF REPLICATE ASSAYS	PLASMA RENIN ACTIVITY ng/ml.3h mean ± SD	COEFFICIENT OF VARIATION %
I	15	12.2 ± 1.6	12.9
II	15	29.9 ± 4.5	15.0
III	13	52.5 ± 6.0	11.4

in a 20 µl aliquot and in a 40 µl aliquot of the Dowex eluate of different plasma samples. The differences were calculated for a lower (0.5 - 2.5) and a higher (2.5 - 10) range of angiotensin I concentrations. The mean difference for the lower range was 0.04 ± 0.18 (n = 32, n.s.) and for the higher range 0.06 ± 0.19 (n = 41, n.s.).

Recovery.

The accuracy of the method was evaluated by adding known amounts of angiotensin I, in a range from 1 to 75 ng, to different plasma samples. In Table 3 the percentage recoveries are presented in relation to the initial amount of angiotensin I present in the plasma samples, and in relation to the amount of angiotensin I added to those samples. In general the percentage recovery increases with the amount of angiotensin I added. There does not seem to be a significant correlation between the percentage recovery and the initial amount of angiotensin I present. Overall, the

recoverv was 87 ± 17 %. A highly significant positive correlation was found between the added amount of angiotensin I and the recovered amount of angiotensin r = 0.98, n = 41, p < 0.001).

Table 3. RECOVERY (%) OF THE RADIOIMMUNOASSAY PROCEDURE A.

RANGE OF ANGIOTENSIN I ADDED ng	RANGE OF PLASMA RENIN ACTIVITIES INITIALLY PRESENT IN THE PLASMA SAMPLES ng/ml.3h			
	0 - 10	10 - 45	45 - 90	0 - 90
0 - 10	91 5 ± 21 5 n - 9	74 0 ± 17 7 n - 5	79.0 ± 13 5 n = 3	83 6 ± 20 5 n = 17
10 - 45	87.5 ± 17 6 n 4	84 2 ± 11 9 n 5	87.0 ± 12 5 n - 10	86 4 ± 12 8 n = 19
45 - 75	-	-	98 2 ± 14 5 n = 5	98 2 ± 14 5 n = 5
0 - 75	90 3 ± 19.7 n - 13	79 1 ± 15 2 n - 10	88 7 ± 14.4 n - 18	87.1 ± 16 6 n - 11

Generation of angiotensin I during incubation.

Eight plasma samples were incubated for zero to 4 hours and the generated amount of angiotensin I was measured (Fig. 2). During the first three hours of incubation a linear generation slope was found in all plasma samples. After 3 hours, a decline in angiotensin I generation was observed in 3 plasma samples. Incubation of all plasma samples at 4° C for 3 hours resulted in a generation of minimal amounts of angiotensin I. A mean of 0.8 ng/ml per 3 h was measured which did not differ significantly from the value before incubation, 0.7 ng/ml per 3 h.

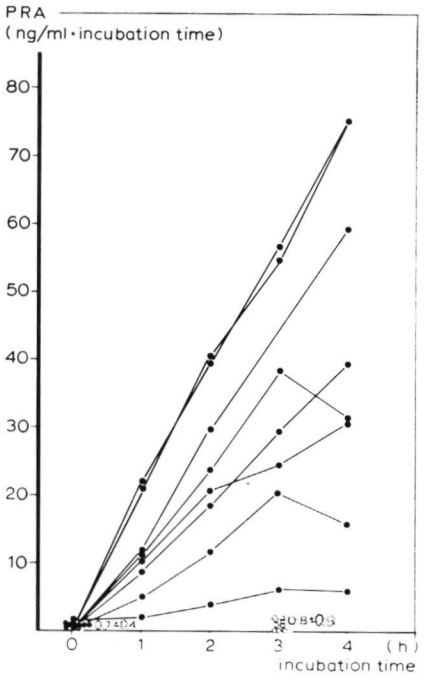

Fig. 2. GENERATION OF ANGIOTENSIN I, MEASURED BY RADIOIMMUNOASSAY (PROCEDURE A). •, PRA MEASURED AFTER INCUBATION AT 37° C FOR DIFFERENT PERIODS, ∘, PRA MEASURED AFTER INCUBATION AT 4° C FOR 3 HOURS.

Differences between angiotensin I standards.

Standard curves as presented in Fig. 3 were obtained when different preparations of the angiotensin I peptide were used. It is clear that the preparations vary widely as far as their immunological properties are concerned. The two batches of the Schwarz Mann preparation and the Beckman standard gave identical results. Compared with these curves those obtained with the Medical Research Council standard and the Sorin preparation differed widely. Angiotensin II,

up to a concentration of 1000 pg per tube, did not displace tracer to any degree.

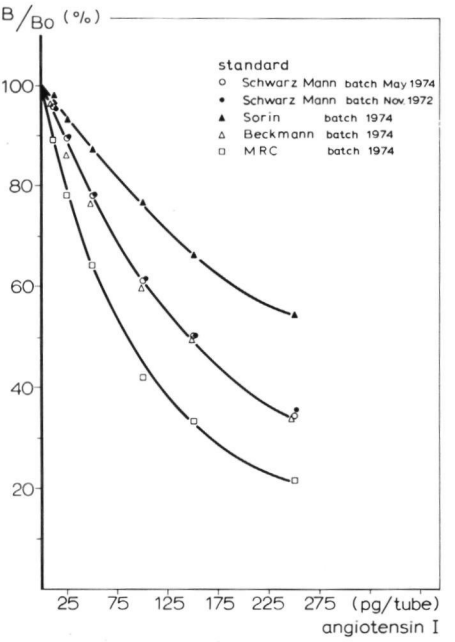

Fig. 3. DIFFERENCES BETWEEN ANGIOTENSIN I STANDARDS. THE STANDARD CURVES WERE ASSAYED WITH RADIOIMMUNOASSAY PROCEDURE A.

Use of different antisera.

The standard curves obtained using two different antisera A and B, are shown in Fig. 4. From the Scatchard plots the dissociation constants of $10 \cdot 10^{-12}$ and $28 \cdot 10^{-12}$ mol/l were calculated for the respective antisera. Fourteen plasma samples were analyzed with the use of these two distinct antisera. The results are presented in Table 4. The correlation between the two series of results was

highly significantly positive ($r = 0.998$, $n = 14$, $p < 0.001$).

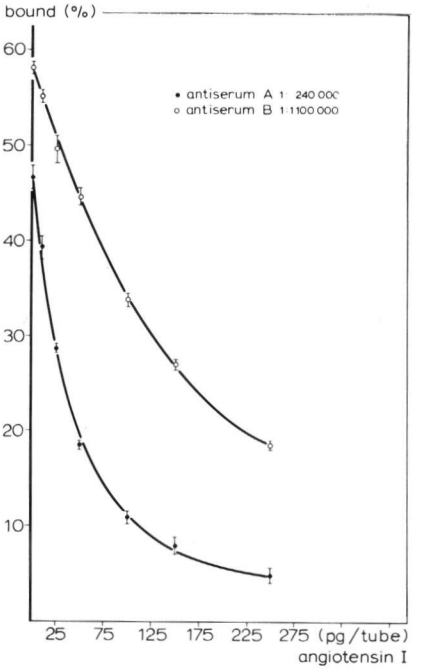

Fig. 4. STANDARD CURVES OBTAINED BY RADIOIMMUNOASSAY PROCEDURE A, USING TWO DIFFERENT ANTISERA.

Calibration.

To compare results obtained by this radioimmunoassay (Procedure A) with those obtained by other assays, two different plasma samples were incubated in the absence and presence of 0.001 and 0.0005 Goldblatt Unit (G.U.) standard renin, respectively. The results are presented in Table 5. The rate of angiotensin I generation (ng/h) by standard renin was almost identical in both plasma samples and increased twice by doubling the amount of renin added. The generated amount of angiotensin I was in both samples

Table 4. PRA VALUES MEASURED BY RADIOIMMUNOASSAY PROCEDURE A USING TWO DIFFERENT ANTISERA.
ANTISERUM A: FINAL DILUTION 1 : 240.000. ANTISERUM B: FINAL DILUTION 1 : 1.100.000. CORRELATION COEFFICIENT r = 0.99, $p < 0.001$.

PLASMA SAMPLE	PRA (ng/ml.3h)	
	ANTISERUM A	ANTISERUM B
1	0	0
2	4.4	6.5
3	7.5	5.0
4	11.4	12.7
5	16.4	15.5
6	26.3	25.3
7	31.4	30.0
8	37.7	35.8
9	40.9	37.5
10	41.3	39.8
11	41.9	45.6
12	42.2	43.1
13	43.3	45.0
14	130.6	138.1

Table 5. RATE OF ANGIOTENSIN I GENERATION BY STANDARD RENIN. THE GENERATED AMOUNTS OF ANGIOTENSIN I WERE MEASURED BY RADIOIMMUNOASSAY PROCEDURE A.

PLASMA SAMPLE *	STANDARD RENIN	
	1/1000 G.U.	1/2000 G.U.
	ANGIOTENSIN I ng/h	
1		
2.5 ml incubated for 3 h	39.3	19.0
5.0 ml incubated for 3 h	34.7	18.5
2		
2.5 ml incubated for 1.5 h	40.6	21.9
2.5 ml incubated for 3 h	38.5	22.1
5.0 ml incubated for 3 h	39.3	19.5

* Rate of angiotensin I generation without standard renin
plasma 1: 15.3 $\pm$ 3.9 ng/ml.h
plasma 2: 6.7 $\pm$ 1.5 ng/ml.h

independent of the volume of the plasma used which excludes substrate deficiency. From the results obtained in the experiment in which plasma sample 2 was incubated for 1.5 and 3 hours it appeared that the rate of angiotensin I generation, expressed as ng/h, was equal for both incubation times and therefore linear with time. The mean value of angiotensin I generation per G.U. was, in terms of the Schwarz Mann Standard $3.9 \pm 0.3 \cdot 10^4$ ng angiotensin per hour.

Concentration of plasma renin.

In 22 normotensive subjects (17 - 55 years) using an unrestricted diet the mean PRA measured after 3 hours of ambulation was 4.6 ± 2.5 ng/ml per 3 h (Schwarz Mann Standard). By dividing the PRA by the rate of angiotensin I generation by standard renin, the mean plasma renin concentration of these normal individuals was calculated to be $0.39 \cdot 10^{-4}$ G.U. renin/ml plasma (range from $0.12 \cdot 10^{-4}$ to $0.91 \cdot 10^{-4}$ G.U./ml).

Radioimmunoassay versus bioassay of PRA.

As was described in the methods section, the bioassay was performed after extraction of angiotensin I onto Dowex suspension and further purification. The final extracts of 24 plasma samples were both radioimmunoassayed and bioassayed. The correlation between the results of the two methods was perfect as is shown in Fig. 5A ($r = 0.92$, $p < 0.001$). The radioimmunoassay values were 2.4 ± 0.6 times higher than those obtained by bioassay. It is emphasized that in the radioimmunoassay, angiotensin I from Schwarz Mann was used as the home standard and in the bioassay the angiotensin II standard from Ciba-Geigy. Comparing both

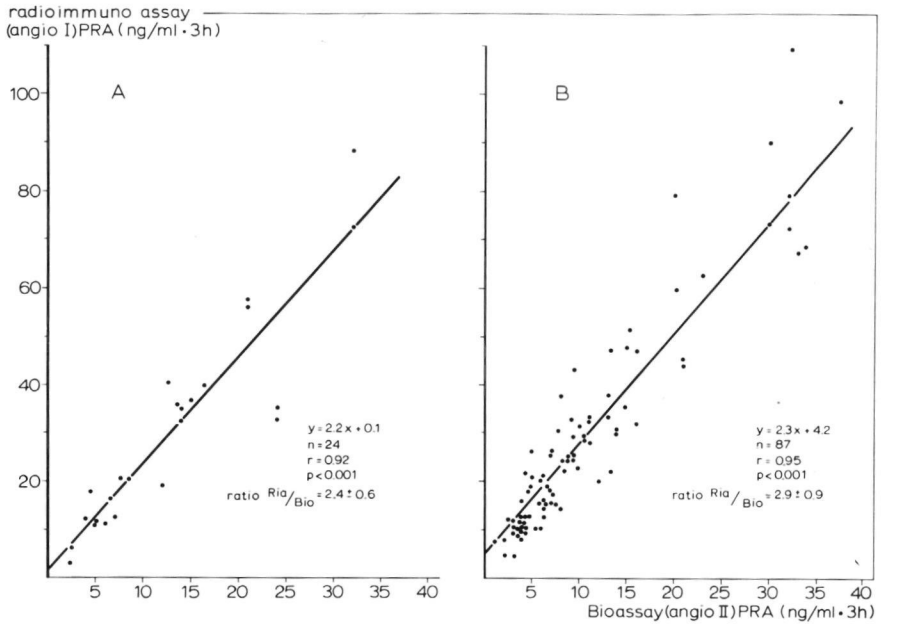

Fig. 5. COMPARISON OF THE RADIOIMMUNOASSAY, PROCEDURE A, WITH THE BIOASSAY.
A. PRA MEASURED IN THE FINAL BIOASSAY EXTRACT WITH BOTH ASSAYS.
B. PRA MEASURED IN THE DOWEX ELUATE BY RADIOIMMUNOASSAY AND IN THE FINAL EXTRACT BY BIOASSAY.

standards with respect to their pressor activity in the rat, angiotensin II appeared to be 2.5 $\pm$ 0.1 times more potent (5 experiments). Therefore, the above mentioned difference between the radioimmunoassay and bioassay values could be completely accounted for by this difference in the pressor activity of these two standard preparations, the commercially available angiotensin I (Schwarz Mann) and the angiotensin II preparation (Ciba-Geigy) used.

From these data it appears that equal results with both the radioimmunoassay and the bioassay can be obtained using

an identical incubation procedure and standard. However, for a routine assay the further purification of the Dowex eluate was too laborious and therefore omitted. In a large series of 87 plasma samples the PRA with this routine assay was compared with the bioassay. The results are presented in Fig. 5B. A highly significant positive correlation was calculated between both methods ($r = 0.95$, $p < 0.001$). The radioimmunoassay values were 2.9 ± 0.9 times higher than those determined by bioassay.

Radioimmunoassay with Dowex extraction (Procedure A) versus a direct radioimmunoassay (Procedure B).

In 27 plasma samples the PRA was measured with both assays. As is shown in Fig. 6 a highly significant positive correlation between the values was found ($r = 0.88$, $p < 0.001$). However, it should be noticed that, roughly, in the lower range of PRA values procedure B gave lower assay results than procedure A, whereas, in the higher range of PRA values procedure B gave equal or higher results than procedure A. The mean ratio between the results of procedure A and procedure B was 1.3 ± 0.5. Moreover, when in the direct assay plasma was incubated for 3 hours at 4° C, a definite amount of angiotensin I or angiotensin I-like activity was generated (6.4 ± 4.2 ng/ml per 3 h) in contrast to the negligible amounts which were measured under these circumstances by procedure A. This amount has to be subtracted from the values obtained after the incubation at 37° C, and this correction was in fact made for the data presented in Fig. 6. A significant positive correlation was found, surprisingly, between generated amounts of angiotensin I after incubation for 3 hours at 37° C and the amounts of angiotensin I or angiotensin I-like activity generated after incubation for 3 hours at 4° C ($r = 0.74$, $p < 0.001$).

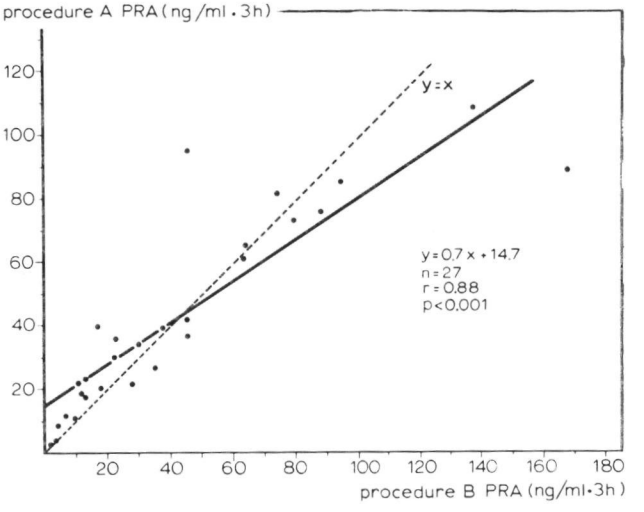

Fig. 6. COMPARISON OF TWO RADIOIMMUNOASSAY METHODS. PROCEDURE A: AN ASSAY USING DOWEX EXTRACTION. PROCEDURE B: A DIRECT ASSAY IN PLASMA.

DISCUSSION

Various authors have pointed out that inherent difficulties can be expected when comparing results of angiotensin measurements by bioassay and radioimmunoassay. It has been reported, as stated in the introduction, that biologically inactive fragments might be immunologically active, or that immunological cross-reacting substances might be present (4.7 - 10). Furthermore, as has been emphasized recently by Oparil and Haber (15): 'Variability in incubation conditions for generating angiotensin and in angiotensin standards has made comparison between bioassay and radioimmunoassay of plasma renin activity difficult to interpret'.

In the present report, studying the reliability of the

measurement of PRA, experiments were performed in which the incubation conditions for generating angiotensin I were identical in both the radioimmunoassay and the bioassay. In our bioassay, however, Asp^1-β-amide-Val^5 Angiotensin II (Ciba-Geigy) was used as the standard which, in the bioassay era, was generally accepted as an unofficial standard (16). In the radioimmunoassay the commercially available Asp^1-Ile^5 angiotensin I (Schwarz Mann) was used as the 'home' standard. The pressor effect of this angiotensin I standard was in our bioassay 0.4 times that of the formerly used angiotensin II, which is in agreement with the findings of Rössler et al. (5). Corrected for this difference in vasopressor activity, the values of PRA with the two methods were indeed in perfect agreement. This conclusion of our study is in agreement with the data Fukuchi et al. (17), Waite et al. (7) and Menard and Catt (8), the last authors using rat plasma, in contradiction, however, with results reported by other authors (1, 4, 5). All authors mentioned here used identical incubation conditions and identical standards in both assay.

As appears from this study the immunoreactivity of various angiotensin I preparations may vary widely which makes comparison of data of different laboratories difficult. Haas and Goldblatt (2), emphasizing that estimates of renin in terms of 'plasma renin activity' are per se unsuitable for comparison studies, recommended the use of an international standard renin. They recalculated the results of a number of published radioimmunoassay procedures using endogeneous substrate in which values were reported for ambulatory normal subjects on unrestricted diets. In terms of Goldblatt Units these values ranged from 0.24 to $0.73 \cdot 10^{-4}$ G.U./ml plasma (mean: $0.4 \cdot 10^{-4}$ G.U./ml plasma). Our normal value, determined by radioimmunoassay, was on average $0.39 \cdot 10^{-4}$ G.U./ml plasma, varying from

$0.12 \cdot 10^{-4}$ to $0.91 \cdot 10^{-4}$ which fits very well into this range. Addition of standard renin to two different plasma samples resulted in angiotensin I generation which was linear with time, with the amount of renin added, and the generated amount was independent of the volume of the plasma used, which demonstrates abundance of substrate present. The reliability of the radioimmunoassay performed with procedure A was further indicated by the observation that the use of two antisera of different sources with different dissociation constants, gave equal values of PRA in a series of 14 plasma samples. Furthermore, incubation of plasma samples obtained from bilaterally nephrectomized patients did not generate measurable quantities of immunologically active substances. The concentrations of angiotensin present, whether 20 or 40 µl of the Dowex eluate were radioimmunoassayed, did not differ significantly.

Several investigators observed a presumably non-specific blank value in the radioimmunoassay of 'non-incubated' plasma samples. The presence of this blank necessitates correction of the 'incubated' value (10, 18, 19). Oparil et al. (20) recently found in plasma samples from anephric patients, after incubation at pH 5.5, for 18 hours in the presence of enzyme inhibitors, a non-renin-related generation of angiotensin I which could make a major contribution to total 'renin activity'. Obviously, by the method described here, this immuno-reactive material was removed by the Dowex extraction. A negligible blank was observed, which is in line with reports of others (4, 7, 8, 10). In procedure B, in which Dowex extraction was not used, a definite blank value was found ranging from 7 to as high as 48 % of the 'incubated' value. After subtraction of the blank value, an overall fair correspondance was observed between the values obtained with both radioimmunoassay procedures A and B. However, on scrutinizing the data of Fig. 6, especially

in the range of the low angiotensin values, method B gave lower values than the radioimmunoassay after Dowex extraction, method A. Because of these considerations a radioimmunoassay in which Dowex is present as the absorbent is used in this laboratory.

ACKNOWLEDGEMENTS

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Chapter II

DETECTION OF LOW-RENIN HYPERTENSION; EVALUATION OF OUT-PATIENT RENIN-STIMULATING METHODS.

Running title: Detection of low renin hypertension.

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The editorial board of the journal 'Clinical Science and Molecular Medicine' decided the plasma renin activity to be expressed in Standard International Units, i.e., $nmol.3\ h^{-1}.l^{-1}$. To enable the comparison of the renin results in this study with those in the other studies of the thesis the renin values in this study have to be multiplied by $\frac{1297}{1000} = 1.297$. The nominator of this factor is the molecular weight of human angiotensin I and the denominator is used in order to express the values after recalculation as $ng.ml^{-1}.3\ h^{-1}$.

SUMMARY

1. Three renin-stimulating methods for detection of low-renin hypertension have been compared. First, renin activity was measured in hospital patients after 5 days of sodium restriction and 3 h ambulation. Secondly, renin activity was measured after frusemide stimulation (0.42 mmol (140 mg) in 18 h) and 3 h ambulation. Thirdly, renin activity was measured after 5 days of chlorthalidone treatment (0.3 mmol (100 mg)/day) and 3 h ambulation. The last two tests were done with the subjects as out-patients without any dietary regimen.

2. In eleven normotensive control subjects and twenty hypertensive patients the results after frusemide were not comparable with those after sodium restriction since the frusemide test did not identify the same renin-suppressed hypertensive subjects as the sodium-restriction procedure.

3. After 5 days of chlorthalidone treatment the renin values in eleven control subjects as well as in thirty-eight hypertensive patients were significantly higher than after sodium restriction. The values obtained after each procedure were closely correlated.

4. Thus the out-patient chlorthalidone procedure identified similar sub-groups of patients as having low- or normal-renin hypertension as did the in-patient sodium-restriction test.

Key words: low-renin hypertension, sodium restriction, frusemide, chlorthalidone.

In fourteen studies, each involving at least fifty hypertensive subjects, the percentage of patients with low-renin hypertension has varied from 12 to 46 (Adlin, Marks & Channick, 1972; Brunner, Sealey& Laragh, 1973; Channick, Adlin & Marks, 1969; Crane, Harris & Nokus, 1972; Creditor & Loschky, 1967; Doyle & Jerums, 1970; Granger, Boucher & Genest, 1968; Gunnels, McGuffin, Robinson, Grim, Wells, Silver & Glenn, 1970; Helmer, 1965; Jerums & Doyle, 1969; Kloppenborg, Benraad, Drayer & Benraad, 1973; Ledingham, Bull & Laragh, 1967; Mroczek, Finnerty & Catt, 1973; Tuck, Williams, Cain, Sullivan & Dluhy, 1973). To identify these low-renin patients widely differing tests have been used. Some investigators have measured basal plasma renin activity whereas others have determined plasma renin activity or plasma renin concentrations after stimulation of renin secretion. When different tests have been used, however, it does not necessarily follow that the same patients will be identified as low- or normal-renin patients.

A widely practised procedure is to measure plasma renin activity after stimulation by rigid sodium restriction and upright posture. This procedure, however, necessitates admission to hospital. For out-patient screening the response of the plasma renin activity to an oral or intravenous dose of frusemide has been recommended (Carey, Douglas, Schweikert & Liddle, 1972; Channick et al., 1969; Dawson & Wallach, 1974; Jose & Kaplan, 1969; Kem, Kramer, Blend, Hemphill, Gomez-Sanchez, White & Kaplan, 1972; Rosenthal, Boucher, Nowaczynski & Genest, 1968). However, according to Brunner et al. (1973) this procedure is not sufficiently discriminating.

In the present study we have measured the renin

response of hypertensive and normotensive subjects to sodium restriction under in-patient conditions. Plasma renin activity values collected in this way are compared with those obtained after two different procedures in which a diuretic drug was administered without restriction of sodium intake, to out-patients to determine whether these tests identify the same populations of normoreninaemic and hyporeninaemic patients.

METHODS

Patients.

Fifty-eight hypertensive patients, whose diastolic blood pressure was, without therapy, above 95 mmHg, were admitted to hospital after antihypertensive treatment had been discontinued for at least 4 weeks. None of the patients had malignant hypertension, and renal and cardiac function was unimpaired. On the fifth day of controlled sodium restriction (15 mmol/24 h) peripheral venous blood for measurement of renin activity was taken at noon after 3 h of ambulation (PRA_{sr}). Eleven young normotensive control subjects, mean age 23.1 $\pm$ 2.4 (SD) years, were subjected to a similar sodium restriction procedure. All were male, with a mean systolic blood pressure, measured after sodium restriction, of 122 $\pm$ 8 mmHg and a mean diastolic blood pressure of 77 $\pm$ 5 mmHg.

The control subjects and a sub-group of twenty hypertensive patients were studied as out-patients after stimulation of renin activity with frusemide (Lasix). They consumed a normal diet and were instructed to take three doses of frusemide orally, i.e. 0.12 mmol (40 mg) 18 h and 12 h, and a last dose, 0.18 mmol (60 mg), 3 h before blood collection. The patients were ambulant during the last 3 h

before blood sampling at noon (PRA_f). The mean age of the sub-group of twenty hypertensive patients was 40.0 ± 8.9 years; nine of them were female and eleven were male. The mean systolic blood pressure, measured after sodium restriction, was 177 ± 21 mmHg and the mean diastolic blood pressure 111 ± 28 mmHg.

After studying the results of renin stimulation with frusemide, plasma renin activity was measured in the same control subjects and in a sub-group of thirty-eight hypertensive out-patients after stimulation with chlorthalidone (Hygroton). After a daily intake of 0.3 mmol (100 mg) of chlorthalidone for 5 days, during which period the patients were on a normal diet, blood was taken at noon, again after 3 h of ambulation (PRA_{chl}). The mean age of the sub-group was 42.6 ± 12.8 years; twenty-four of them were female and fourteen were male. The mean systolic blood pressure, measured after sodium restriction, was 166 ± 24 mmHg and the mean diastolic blood pressure was 110 ± 15 mmHg. The sub-groups of hypertensive patients did not differ significantly in respect of mean age, sex or blood pressure.

In ten of the normotensive control subjects plasma renin activity was measured on the third, sixth and seventh day as well as on the fifth day of chlorthalidone medication.

In eight hypertensive patients renin activity was measured on the fifth day of chlorthalidone treatment and after long-term medication (more than 42 days) with chlorthalidone alone. Blood for renin measurements was collected at noon after 3 h of ambulation. During chlorthalidone medication the control subjects and the hypertensive patients were on a normal diet.

The hypertensive patients were chosen on a random basis. Both normotensive control subjects and the hypertensive patients gave their informed consent to the studies.

Analytical methods.

In this study, plasma renin activity (PRA) is defined as the amount of angiotensin I formed after an incubation procedure as described by Boucher, Veyrat, de Champlain & Genest (1964). Blood for renin measurement was collected in ice-cooled containers. EDTA was used as an anticoagulant. The amount of angiotensin I in the eluate of the Dowex column was measured by radioimmunoassay.

The antiserum was raised in a rabbit against angiotensin I. Final antiserum dilution was 1 : 1 100 000. Iodinated angiotensin I, obtained from Sorin (I.R.E. Mol Belgium), was used without further purification. Standard angiotensin I was obtained from Schwarz Mann, Orangeburg, New York. The assays were carried out by means of a double-antibody solid-phase technique. This method of PRA measurement has an intra-assay variation of 7.8 - 9.1 % and an interassay variation of 10.4 - 15.0 % for different renin activities. The recovery of angiotensin I was 108 $\pm$ 11 %.

Plasma electrolytes were measured by flame photometry with internal standard.

Non-parametric statistical methods were used to detect differences between two groups (Wilcoxon's two-sample test) and to calculate correlation coefficients between variables (Spearman's correlation coefficient r). Results are expressed as means $\pm$ SD.

RESULTS

Plasma renin activity in normotensive control subjects.

The mean of the PRA values after sodium restriction (PRA_{sr}) (19.0 $\pm$ 10.0 nmol.3h^{-1}.l^{-1}) did not differ signifi-

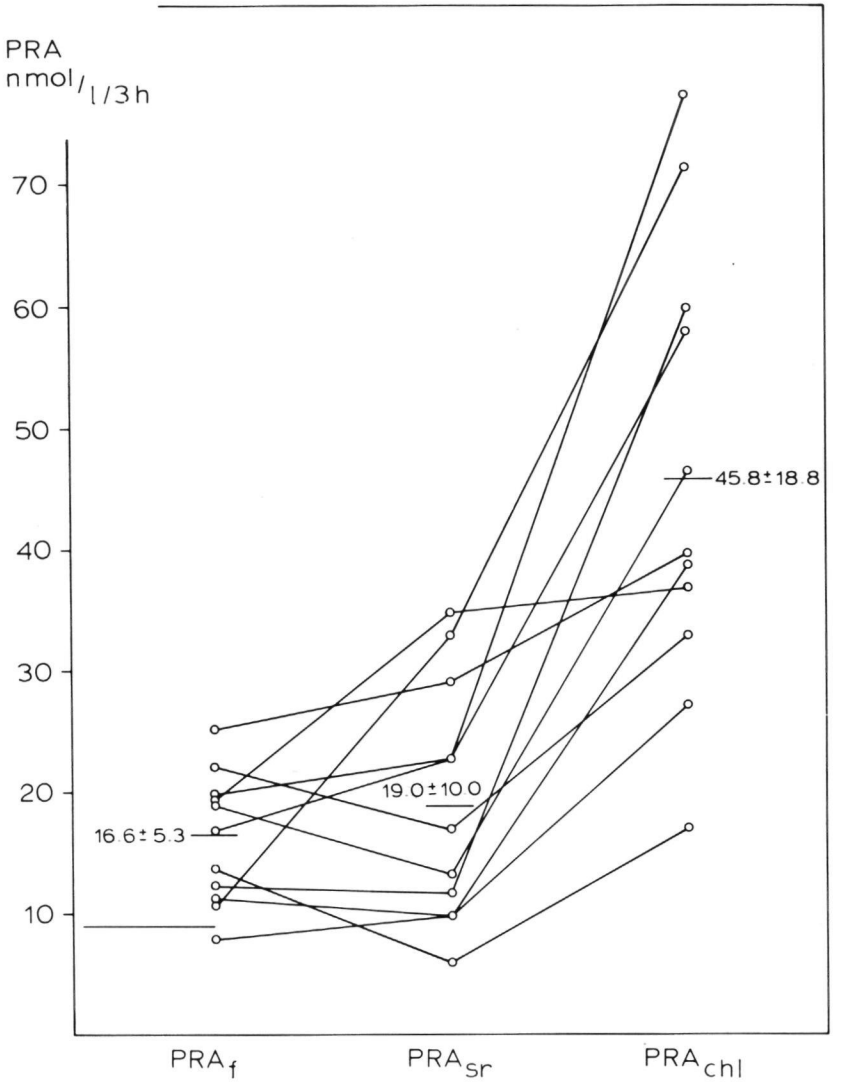

Fig. 1. RELATION BETWEEN PRA_{sr}, PRA_{f} AND PRA_{chl} IN 11 NORMOTENSIVE CONTROL SUBJECTS.

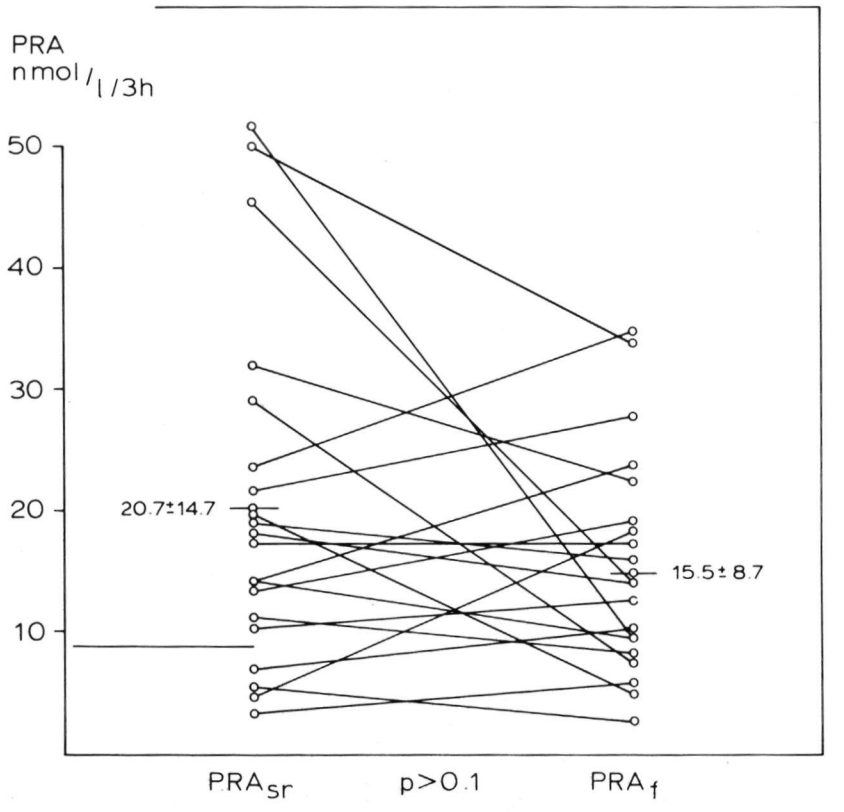

Fig. 2. RELATION BETWEEN PRA$_{sr}$ AND PRA$_f$ IN 20 PATIENTS WITH HYPERTENSION.

cantly from the mean of those after frusemide (PRA_f) (16.6 ± 5.3 nmol.3 $h^{-1}.l^{-1}$), but was highly significantly ($P < 0.005$) different from the mean of those after chlorthalidone (PRA_{chl}) (45.8 ± 18.8 nmol.3 $h^{-1}.l^{-1}$) (Fig. 1). It should be noted that the effect of frusemide stimulation is variable, as five PRA_f values are equal to or lower than, and six are higher than, PRA_{sr} values. By contrast, the PRA_{chl} values were consistently higher than the corresponding PRA_{sr} and PRA_f values. One of the PRA_{sr} values in the normotensive control group was below the normal range measured in a larger group of normotensive subjects. The corresponding PRA_{chl} value was also lower than that of the other subjects, whereas his PRA_f value falls within the range of the other ten normotensive controls.

Plasma renin activity in hypertensive patients.

The mean of the PRA_{sr} values (20.7 ± 14.7 nmol.3 $h^{-1}.l^{-1}$) did not differ significantly from the mean PRA_f value (15.5 ± 8.7 nmol.3 $h^{-1}.l^{-1}$) (Fig. 2). The correlation between PRA_{sr} and PRA_f was not significant ($r = + 0.29$, $n = 20$, $P > 0.1$). As in the normotensive controls there was no parallelism between the PRA_{sr} and the corresponding PRA_f values. The PRA_f values of twelve patients were equal to or lower (remarkably lower in some) than their corresponding PRA_{sr} values, whereas in eight patients the PRA_f values were higher than their PRA_{sr} values.

With the response to sodium restriction used as a criterion of low- and normal-renin hypertensive patients, the frusemide test resulted in misclassification in five cases.

The mean of the PRA_{chl} values (29.1 ± 25.5 nmol.3 $h^{-1}.l^{-1}$) was significantly ($P < 0.001$) higher than the

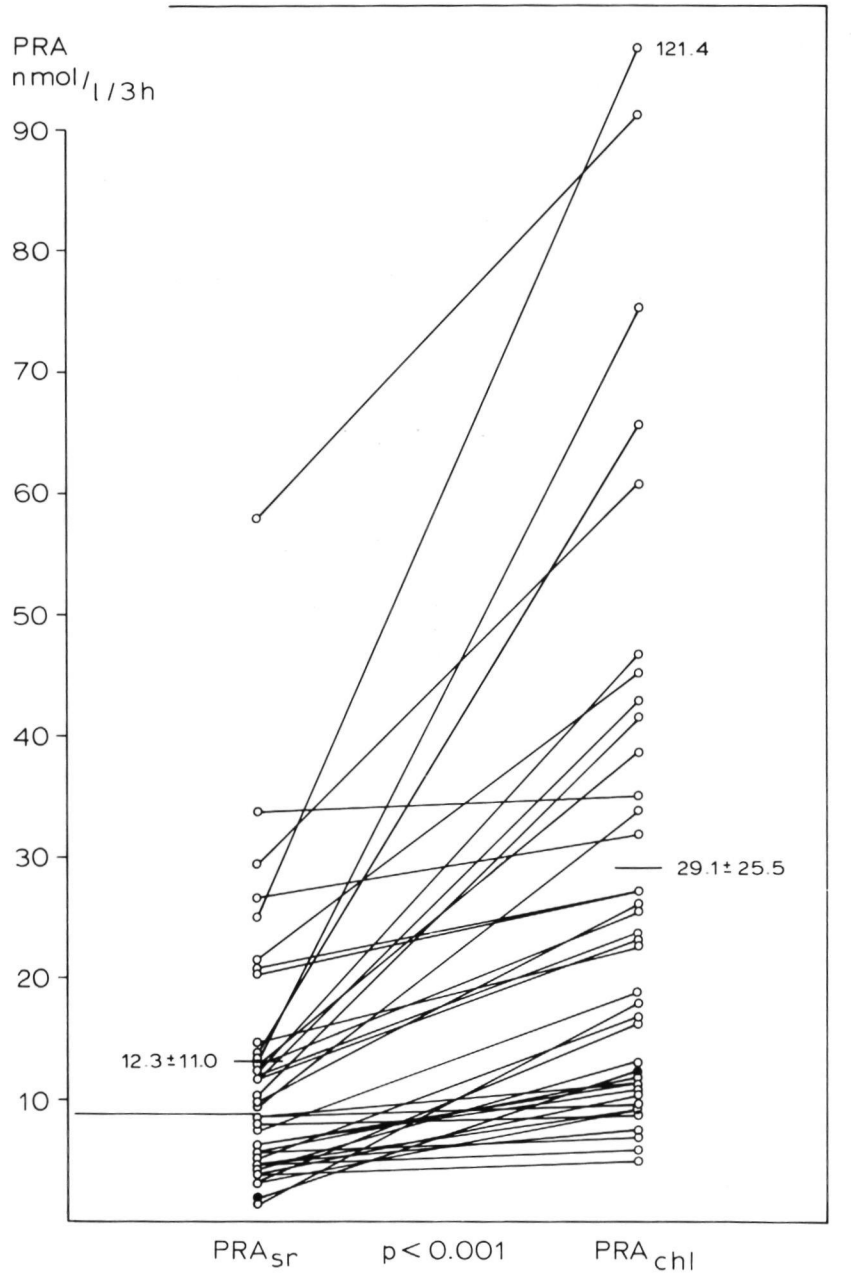

Fig. 3. RELATION BETWEEN PRA_{sr} AND PRA_{chl} IN 38 PATIENTS WITH HYPERTENSION. •, RESULTS FOR PATIENT WITH PRIMARY ALDOSTERONISM.

mean of the PRA_{sr} values (12.3 ± 11.0 nmol.3 $h^{-1}.l^{pl}$), and the PRA_{chl} values were without exception higher than the corresponding PRA_{sr} values (Fig. 3). In this group of thirty-eight patients there was a highly significant positive correlation between PRA_{chl} and PRA_{sr} ($r = + 0.78$, $P < 0.001$).

If these hypertensive patients are divided into those with hyporeninaemia and those with normal plasma renin activity according to their PRA_{sr} values, eighteen would belong to the first and twenty to the second group. The PRA_{chl} values obtained in the first group ranged from 5.1 to 18.8 nmol.3 $h^{-1}.l^{-1}$, and in the second from 22.9 to 121.4 nmol.3 $h^{-1}.l^{-1}$. The difference,therefore, between the highest value in the low-renin group and the lowest value in the normal-renin group, was 4.1 nmol.3 $h^{-1}.l^{-1}$, which was about 4.5-fold the difference of 0.9 nmol.3 $h^{-1}.l^{-1}$ between the highest PRA_{sr} value in the low-renin group and the lowest PRA_{sr} value in the normal-renin patients. Thus, with renin values after sodium restriction used as a criterion for low and normal renin, the chlorthalidone test did not misclassify any of the thirty-eight patients.

Plasma renin activity after chlorthalidone treatment during various periods.

To determine whether the duration of diuretic therapy was critical, plasma renin activity was measured in ten normotensive control subjects on the third, fifth, sixth and seventh days of chlorthalidone medication. The mean PRA_{chl} value on the third day was 29.6 ± 16.2 nmol.3 $h^{-1}.l^{-1}$, significantly ($P < 0.05$) lower than the mean PRA_{chl} on the fifth day (48.0 ± 24.0 nmol.3 $h^{-1}.l^{-1}$). PRA was measured in four subjects on the sixth day and in six subjects on the seventh day. In view of the small number of subjects these

PRA values were taken together. The mean PRA value thus obtained was lower (34.9 ± 14.3 nmol.3 $h^{-1}.l^{-1}$) than that on the fifth day, but not significantly so.

Renin activity was measured in eight hypertensive patients on the fifth day of chlorthalidone treatment and after long-term medication (more than 42 days) with chlorthalidone alone. The mean plasma renin activity on the fifth day was significantly ($P < 0.03$) higher (39.1 ± 14.8 nmol. 3 $h^{-1}.l^{-1}$) than that after long-term treatment (19.2 ± 14.5 nmol.3 $h^{-1}.l^{-1}$). Indeed, five of the eight PRA values measured after long-term medication were in the range of the PRA_{chl} values of the eighteen patients with low-renin hypertension and only one patient had a low PRA after chlorthalidone therapy for 5 days as well as after sodium restriction and upright posture.

Plasma potassium values.

In all normotensive subjects as well as in some of the hypertensive patients blood was taken for renin and plasma potassium determination. In the eleven normotensive control subjects the mean plasma potassium value was 3.83 ± 0.51 mmol/l after 5 days of sodium restriction. After frusemide administration the mean potassium value was lower (3.77 ± 0.26 mmol/l) but not significantly so. After 5 days of chlorthalidone treatment the mean potassium value was significantly ($P < 0.005$) lower than after both the sodium restriction and the frusemide test (3.23 ± 0.26 mmol/l).

In twenty-five hypertensive patients the mean plasma potassium value after chlorthalidone medication for 5 days was 3.08 ± 0.42 mmol/l; this was significantly ($P < 0.005$) lower than that after sodium restriction in these patients (4.04 ± 0.41 mmol/l) and also significantly ($P < 0.05$) lower than the mean plasma potassium value in nine patients after

frusemide (3.59 ± 0.45 mmol/l).

Adverse reactions.

After frusemide severe adverse reactions were noted in four subjects, i.e. severe tetany, hypotension, fainting and severe hypokalaemia (1.9 mmol/l). The patient with severe hypokalaemia had low potassium values (less than 3.0 mmol/l) before treatment. After chlorthalidone medication the lowest plasma potassium observed was 2.4 mmol/l. This patient had no complaints during this test. This patient had low PRA and hyperaldosteronism. At operation an adenoma of one adrenal was found. In the other patients with low-renin hypertension no evidence of primary aldosteronism was found.

Milder complaints were noted in four other patients (nausea, vomiting, dyspnoea and tiredness). Adverse reactions were thus noted in eight of the thirty-five frusemide tests. Chlorthalidone was administered fifty-four times; one subject complained of mild tetany, one of dizziness and some about tiredness. No severe adverse reactions were noted.

DISCUSSION

Carey et al. (1972) used frusemide in three doses over a period of 18 h and observed two separate groups of hypertensive patients, one of which had suppressed and the other normal plasma renin activity. The present study, using practically the same procedure as Carey, yielded PRA values, which, in twelve out of twenty hypertensive patients and seven out of eleven normotensive control subjects, were equal to or lower than the corresponding PRA values after sodium restriction.

This unpredictable effect of frusemide has also been noted by Brunner et al. (1973), who observed lack of renin response after intravenous administration of frusemide in a number of hypertensive patients and did not regard this test as adequate for the identification of renin sub-groups. A subnormal response to a single oral dose of frusemide with a normal increase of renin activity after sodium restriction has also been observed by Channick et al. (1969) and Adlin et al. (1972). Conversely, patients with subnormal response to sodium restriction may respond normally to a single dose of frusemide (Channick et al., 1969).

The data reported in this study and those reported by Channick et al. (1969) clearly indicate that the frusemide test does not discriminate the same population of 'low-renin' patients as the sodium restriction test. By contrast all the PRA values obtained from hypertensive patients and control subjects after chlorthalidone medication (PRA_{chl}) are higher than the corresponding renin values measured after sodium restriction (PRA_{sr}). Furthermore, all hypertensive patients who, according to their renin values on sodium restriction, are classified as 'low-renin hypertensive' show PRA_{chl} values which are lower than the lowest PRA_{chl} value measured in the normal PRA_{sr} group. All the PRA_{chl} values of the low PRA_{sr} hypertensive patients are lower than the PRA_{chl} values measured in the normotensive control subjects with a normal PRA_{sr}. It should be added that in both normotensive control subjects and in hypertensive patients the number of days of treatment with chlorthalidone is of importance. Thus plasma renin activity after long-term chlorthalidone administration was significantly lower than after the chlorthalidone test. This may be of relevance in the controversy surrounding the renin response to long-term diuretic therapy.

Our findings indicate that this out-patient test

discriminates the same population of hyporeninaemic patients as does the most widely accepted clinical test in which a rigidly controlled low sodium diet is combined with 3 h of ambulation.

To define a normal range of PRA_{chl} values it is necessary to measure this variable in a larger sample of normotensive control subjects, closely matched to the hypertensive population. This study is now proceeding in our laboratory.

ACKNOWLEDGEMENTS

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Chapter III

DETECTION OF LOW-RENIN HYPERTENSION; COMPARISON OF PLASMA RENIN ACTIVITY AFTER CHLORTHALIDONE WITH THE RESULTS AFTER DIFFERENT LEVELS OF SODIUM INTAKE

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SUMMARY

The use of renin stimulation with chlorthalidone to detect low-renin hypertension has been further evaluated in this study. The classification of patients, according to the chlorthalidone test, as low- or normal-renin hypertensives has been compared with the classification of the same patients according to the plasma renin activity measured after a moderate, normal or high sodium intake.

In 60 patients, the data of 38 of them have been reported in a previous study, the plasma renin activity was measured both after the chlorthalidone test as well as after sodium restriction in hospital. Two patients were misclassified as low- or normal-renin according to one of the tests.

The patients with low plasma renin activity after chlorthalidone did not have lower renin values after a moderate, unrestricted or high sodium intake than those with normal renin activity after chlorthalidone. Moreover, the plasma renin activities found after a moderate or unrestricted sodium intake in these low-renin patients, were all within the range of the renin values found in normotensive control subjects after a comparable sodium intake.

The chlorthalidone test is a reliable out-patient procedure for the detection of low-renin hypertension. The test is a useful substitute for the in-patient sodium restriction test.

Dividing hypertensive patients in hypo- and normoreninemics according to the plasma renin activity measured after a moderate or high sodium intake resulted in a classification, radically different from that obtained with the chlorthalidone test. Adequate renin stimulating maneuvers are needed for a reliable identification of a low-renin state.

INTRODUCTION

As was mentioned earlier, widely differing tests have been used to identify low-renin patients (1). Some investigators used a procedure in which renin secretion is stimulated by ambulation without sodium depletion (2 - 6). Brunner et al (7) related the plasma renin activity (PRA) measured after ambulation without sodium restriction or depletion to urinary sodium excretion. Other investigators used more rigorously stimulating methods as either sodium restriction (8 - 17) or pharmacologically induced stimulation of renin secretion by furosemide (18 - 20), diazoxide (21) or hydralazine (22).

The low-renin discriminating capacity of three renin stimulating maneuvers has been compared in a previous study (1). Controlled dietary sodium restriction and short-term chlorthalidone medication appeared to detect the same hypertensive individuals as low-renin patients. Administration of furosemide did not identify the same patients as low-renin hypertensives as did the two other procedures.

In this study the results of the chlorthalidone procedure have been compared with the results of renin measurements in normotensive and hypertensive subjects on moderate, unrestricted and high sodium intake.

METHODS

First, in 22 hypertensive patients (11 male and 11 female, age range from 21 - 57 years), the PRA measurements after short-term chlorthalidone medication were compared with the PRA measured after 5 days of controlled sodium restriction in hospital. The study design has been described earlier (1).

Secondly, the PRA was measured in 22 normotensive (diastolic blood pressure lower than 90 mmHg) control subjects (12 females, 10 males age range 17 to 55 years) at noon after normal activity in the morning hours. Thereafter the PRA was measured on the fifth day of chlorthalidone medication (100 mg per day at 8.00 a.m.) again at noon after 3 h of ambulation. These subjects (hospital personnel and their relatives) consumed a normal diet. The urinary sodium excretion was measured in urine collected during the 24 hours previous to blood sampling for PRA. Using the same two tests, PRA measurements were also done in 15 hypertensive (diastolic blood pressure higher than 90 mmHg) patients (7 females, 8 males, age range 25 to 58 years). In a second group of hypertensive patients (8 females, 7 males, age range 32 to 58 years) the results of the chlorthalidone test were compared in the same way with renin measurements after five days during which an unrestricted sodium diet was supplemented with 150 mEq sodium chloride in divided doses orally. All these hypertensive patients were off antihypertensive treatment for at least four weeks.

The PRA was measured by radioimmunoassay (23).

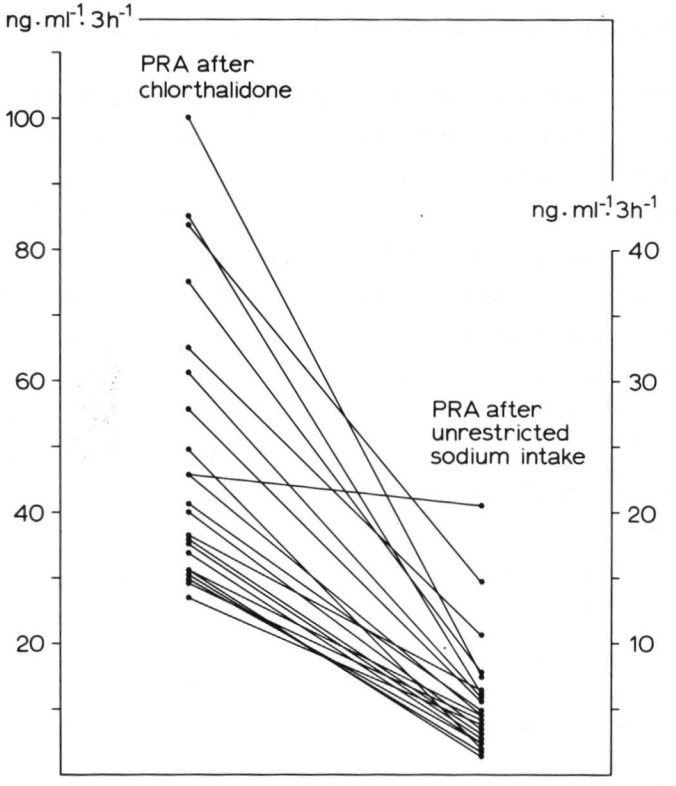
A non parametric test was used to calculate correlation coefficients (Spearman's rank correlation test). The results are expressed as means $\pm$ SD.

Comparison of PRA values measured after sodium restriction (15 mEq/24 h) with those measured after the chlorthalidone test in 22 patients with benign essential hypertension.

According to the PRA measured after sodium restriction and chlorthalidone in normotensive control subjects (Table 1) 5 of 22 hypertensive patients had low-renin values after sodium restriction (range 4.4 to 10.9 $ng.ml^{-1}.3\ h^{-1}$) and 17 had a normal PRA (range 11.5 to 40.6 $ng.ml^{-1}.3\ h^{-1}$). In four of these low-renin hypertensives the PRA measured after chlorthalidone was lower than the lowest PRA found in the control subjects (range 7.5 to 25.0 $ng.ml^{-1}.3\ h^{-1}$). The fifth patient, with a PRA after sodium restriction of 10.9 $ng.ml^{-1}.3\ h^{-1}$, had a PRA after chlorthalidone within the range of the normotensive subjects (34.7 $ng.ml^{-1}.3\ h^{-1}$). All but one of the 17 hypertensive patients with a PRA after sodium restriction within the range of the normotensive subjects, had normal PRA values after chlorthalidone (range 26.9 to 85.0 $ng.ml^{-1}.3\ h^{-1}$). One patient had a normal PRA after sodium restriction (13.1 $ng.ml^{-1}.3\ h^{-1}$) and a low renin value after chlorthalidone (20.1 $ng.ml^{-1}.3\ h^{-1}$).

Comparison of PRA values measured after an unrestricted sodium diet with those after the chlorthalidone test in 22 normotensive control subjects.

The PRA values measured after the chlorthalidone test in 22 normotensive control subjects ranged from 26.9 to 100.0 $ng.ml^{-1}.3\ h^{-1}$, mean 48.4 $\pm$ 21.2 $ng.ml^{-1}.3\ h^{-1}$. The corresponding PRA values without chlorthalidone were on average 5.7 $\pm$ 4.6 $ng.ml^{-1}.3\ h^{-1}$with a range from 1.4 to 20.6 $ng.ml^{-1}.3\ h^{-1}$ (Fig. 1). The correlation between PRA values measured after both procedures was highly signifi-



'ig. 1. COMPARISON OF PRA AFTER CHLORTHALIDONE WITH PRA
.FTER AN UNRESTRICTED SODIUM INTAKE IN 22 NORMOTENSIVE
'ONTROL SUBJECTS.

Table 1. RANGES OF PLASMA RENIN ACTIVITIES MEASURED AFTER 3 H OF AMBULATION AT NOON AND OF THE URINARY SODIUM EXCRETION DURING 24 HOURS STARTING AT NOON ON THE PREVIOUS DAY IN NORMOTENSIVE CONTROL SUBJECTS.

	PRA	URINARY SODIUM EXCRETION
	$ng.ml^{-1}.3\ h^{-1}$	mEq/24h
SODIUM RESTRICTION TEST n = 26	11.5 - 59.5	2 - 20
CHLORTHALIDONE TEST n = 22	26.9 - 100.0	78 - 320
UNRESTRICTED SODIUM DIET n = 22	1.4 - 20.6	67 - 284

cantly positive ($r = 0.70$, $p < 0.001$). The PRA values in normotensive control subjects after controlled sodium restriction in hospital, after an unrestricted sodium intake and after the chlorthalidone test on an out-patient base are shown in table 1 with the urinary sodium excretion during the 24 hours previous to the renin measurement.

Comparison of PRA values after moderate sodium intake (115 mEq/24h) in hospital or on an out-patient base with unrestricted diet with those after the chlorthalidone test in 15 hypertensive patients.

After moderate sodium intake the PRA values ranged from 1.4 to 11.6 $ng.ml^{-1}.3\ h^{-1}$. In the 8 patients in which the PRA was measured in hospital on moderate sodium intake the PRA ranged from 1.5 to 11.6 $ng.ml^{-1}.3\ h^{-1}$ and in the 7 patients with unrestricted sodium intake on an out-patient

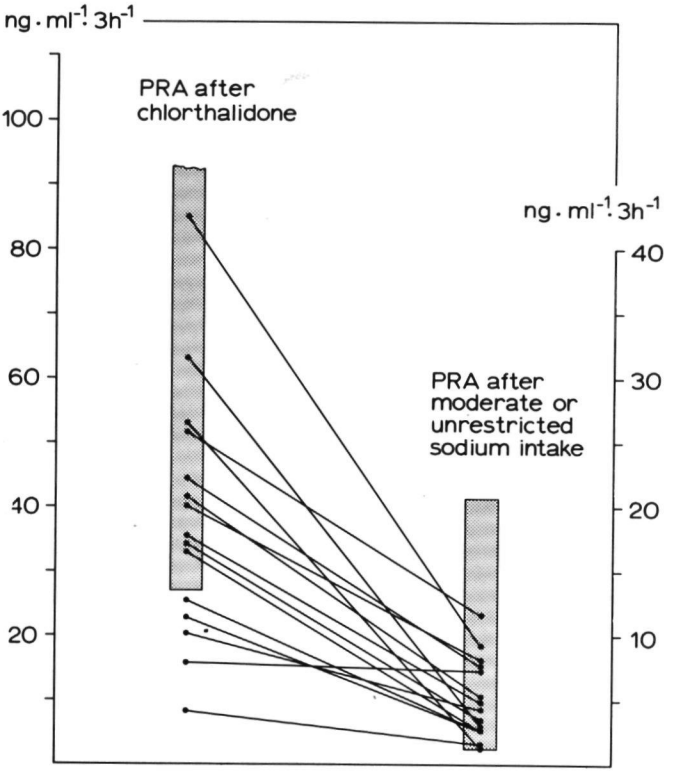


Fig. 2. COMPARISON OF PRA AFTER CHLORTHALIDONE WITH PRA AFTER MODERATE OR UNRESTRICTED SODIUM INTAKE IN 22 HYPERTENSIVE PATIENTS. THE SHADED AREA REPRESENTS THE RANGE IN NORMOTENSIVE CONTROL SUBJECTS.

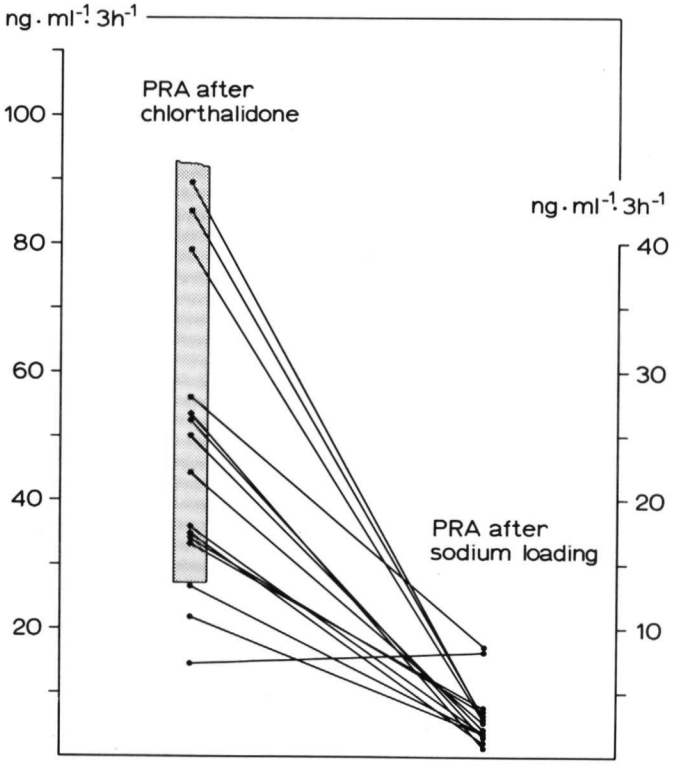


Fig. 3. COMPARISON OF PRA AFTER CHLORTHALIDONE WITH PRA AFTER SODIUM LOADING IN 15 HYPERTENSIVE PATIENTS. THE SHADED AREA REPRESENTS THE RANGE IN NORMOTENSIVE CONTROL SUBJECTS.

base from 1.4 to 9.1 ng.ml^{-1}.3 h^{-1}. The corresponding renin activities after the out-patient chlorthalidone test ranged from 8.4 to 85.0 ng.ml^{-1}.3 h^{-1}(Fig. 2).

Five out of these 15 patients had PRA values after chlorthalidone lower than the lowest value found in normotensive control subjects (range 8.4 to 25.3 ng.ml^{-1}.3 h^{-1}). The PRA values measured on moderate or unrestricted sodium intake in these 5 patients were all within the range of the values measured on an unrestricted sodium intake in normotensive control subjects (range 1.5 to 7.4 ng.ml^{-1}.3 h^{-1}). The correlation coefficient between the corresponding PRA values measured after both procedures in this group of hypertensive patients was not significant ($r = 0.34$, $p > 0.1$).

Comparison of PRA values after sodium loading with those after the chlorthalidone test in 15 hypertensive patients.

In this group of patients the PRA measured after sodium loading (unrestricted sodium intake and 150 mEq of sodium chloride per day in addition) ranged from 0.6 to 8.6 ng.ml^{-1}.3 h^{-1} and the corresponding PRA values after chlorthalidone from 14.5 to 85.0 ng.ml^{-1}.3 h^{-1}(Fig. 3).

Three out of these 15 patients had PRA values after chlorthalidone lower than the lowest value found in normotensive control subjects (14.5, 21.9 and 26.8 ng.ml^{-1}.3 h^{-1}). The corresponding renin values measured after sodium loading were 8.1, 2.0 and 1.9 ng.ml^{-1}.3 h^{-1} respectively). The two patients with the lowest renin values after sodium loading (0.6 and 1.0 ng.ml^{-1}.3 h^{-1}) had normal renin values after chlorthalidone (53.8 and 34.7 ng.ml^{-1}.3 h^{-1} respectively). The correlation coefficient between the corresponding PRA values measured after both procedures in this group of 15 hypertensive patients was not significant ($r = - 0.03$, $p > 0.1$).

The frequency at which low renin hypertension has been reported to occur differs widely, from 12 - 46 % (1). To explain this wide range at least two questions have to be answered. First, what is the range of renin values in normotensive control subjects under comparable circumstances and secondly, do all tests indeed identify the same patients as low-renin hypertensives. In a previous study of this laboratory (1) at least the chlorthalidone test and the sodium restriction test appeared to identify the same patients as low-renin or normal-renin hypertensives. Dividing a group of hypertensive patients according to the furosemide test a radically different subclassification was obtained in that study.

In this study the range of PRA values in a relatively small sample of normotensive control subjects has been given for the sodium restriction test and the chlorthalidone test. The range of renin values in normotensive control subjects as well as in hypertensive patients is well known to vary widely. Therefore, accepting a range from observations in a relatively small number of subjects or patients is rather arbitrary. With these limitations in mind, the given ranges have been used to classify hypertensive patients in hypo- and normo reninemics. From the results obtained in an earlier study (1), none of 38 hypertensive patients were misclassified by either test. In the additional 22 hypertensive patients in this study 20 of the 22 patients again showed renin values after either method in the low or normal range. Therefore, in 58 out of 60 patients the same classification as hypo- or normo reninemics was obtained by both the sodium restriction as well as the chlorthalidone test. One of the remaining two patients had a PRA after sodium restriction (10.9 $ng.ml^{-1}.3\ h^{-1}$) just

below the normal range and a PRA after chlorthalidone (34.7 $ng.ml^{-1}.3\ h^{-1}$) in the lower part of the range in normotensive control subjects. The other patient had a just normal PRA after sodium restriction (13.1 $ng.ml^{-1}.3\ h^{-1}$) and a PRA after chlorthalidone of 20.1 $ng.ml^{-1}.3\ h^{-1}$, slightly lower than the lowest value found in normotensive control subjects (26.9 $ng.ml^{-1}.3\ h^{-1}$). The correlation coefficient between the renin values obtained in either procedure was as high as + 0.77 ($n = 60$, $p < 0.001$). The correlation coefficient between the results of both procedures in the earlier report (1) was + 0.78! ($n = 38$, $p < 0.001$).

From the results depicted in Fig. 2 and 3 it appears that, neither after a moderate or normal sodium intake, nor after sodium loading, the same patients were identified as hypo- or normoreninemics as with the chlorthalidone test. Moreover, there appeared to be a huge overlap between the range of renin values measured in normotensive control subjects after an unrestricted sodium intake and the range found in - according to the chlorthalidone test - low-renin hypertensive patients after a moderate, an unrestricted or even a high sodium intake.

This study therefore confirms the conclusion of an earlier study in this laboratory (1), that the chlorthalidone test is a reliable out-patient procedure to detect low-renin hypertension. In addition it has been shown that this test, as well as the in-patient sodium restriction test give a radically different renin subclassification than that obtained by measuring the PRA in hypertensive patients on a moderate, an unrestricted or a high sodium intake. Therefore, in order to discriminate relative suppression of renin activity an adequately stimulating procedure seems necessary.

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Chapter IV

AUTOMATED BLOOD PRESSURE RECORDING VERSUS CONVENTIONAL MANOMETRY; COMMENTS ON BLOOD PRESSURE VARIABILITY.

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In this study blood pressures measured by auscultation with a standard sphygmomanometer were compared with blood pressures recorded with an automated device. In the first part of the study the systolic blood pressure was measured simultaneously by auscultation and the automated device. The difference between both readings was not significant and a highly significant positive correlation was found between both systolic blood pressures.

However, because of the absence of a physician or nurse during the automated blood pressure recordings, the blood pressures recorded during 40 minutes of recumbency were significantly lower than the pressures measured by auscultation during another 40 minutes of recumbency, irrespective of the sequency of these maneuvers.

The mean of the systolic and diastolic blood pressures recorded during 30 minutes of recumbency were both in hypertensive patients and normotensive control subjects about 7 % lower than the corresponding casual blood pressures. The lowest systolic and diastolic blood pressure recorded during that period was in patients and control subjects about 12 % lower than the casual auscultatory blood pressure.

A more marked decrease in systolic and diastolic blood pressure was observed during the night. The lowest systolic blood pressure recorded during the night in hypertensive patients and normotensive control subjects was about 32 % lower than the casual systolic blood pressure at day-time. With regard to the diastolic blood pressures, the relative decrease was significantly less in the hypertensive patients than in the control subjects (26 and 35 % respectively, $p < 0.003$).

The variability of the blood pressure was defined as

the difference between the day-time casual blood pressure, measured by auscultation, and the lowest blood pressure recorded at night with the automated device.

The variability was not significantly correlated with the age of the normotensive control subjects or the hypertensive patients. In the group of patients with benign essential hypertension a loose though significant negative correlation was calculated between the plasma renin activity and the variability of the blood pressure.

From the correlation coefficients found in normotensive control subjects and hypertensive patients between the casual blood pressure at day-time, the lowest blood pressure recorded at night and the variability, the conclusion seemed inevitable that the casual systolic and diastolic day-time blood pressure levels in normotensive control subjects are per se more varying qualities than those in hypertensive patients.

The use of an automatic blood pressure monitor prevents observer's bias in the evaluation of blood pressure responses to different stimuli or therapeutic regimens. Moreover, errors as terminal digit preference are overcome. However, these and other measurement errors account for only a minor part of the total variability of the results of assessing the blood pressure. The factors inducing a greater variability in blood pressure measurement include environmental conditions; e.g. the reported differences between the casual and basal blood pressure and the blood pressure decrease during sleep (1, 2, 3).

The significance of the total variability or 'response instability' of the diastolic blood pressure has been shown clearly by Varady and Maxwell (4). These investigators collected data on the diastolic blood pressure by standard manometry in 2442 hospitalized patients with mean diastolic blood pressures ranging from 60 to 178 mmHg (about 27.000 measurements). The blood pressure was taken four times daily, during three days, by different observers. The range of the differences found in the whole group between the lowest and highest diastolic blood pressure measured in each patient ranged from 0 to 90 mmHg with a mean of 31 mmHg.

In order to assess the variability of the blood pressure as a physiological phenomenon and to study the antihypertensive effect of different pharmacological agents an automated device, excluding observer's bias and terminal digit preference, would be of great value. In this study blood pressures measured by standard manometry and with an automated device have been compared.

The automatic blood pressure monitor used was the Arteriosonde® 1216 (Roche Medical Electronics Division, Hoffmann - La Roche Inc., Orangeburg, N.Y., U.S.A.). With this device systolic and Korotkoff, fourth-phase diastolic pressures are measured with ultrasound detection of pulsatile movements of the brachial artery wall as it is relieved from compression by a pneumatic cuff. The results are recorded as point plots on a connected Arteriocorder® 1508.

A portable model of a standard mercury sphygmomanometer, Erkameter 300, was used to measure the blood pressure by auscultation. Fourth phase diastolic blood pressures were read.

In ten normotensive volunteers the blood pressure was measured by standard manometry as well as by automatic recording with the Arteriosonde®. By each procedure the blood pressure was taken every ten minutes during one of two successive hours of recumbency at day-time. In five subjects the blood pressure was measured by auscultation during the first hour and recorded with the Arteriosonde® in the second hour, and in the remaining subjects the reverse sequence was followed. The physician, present for measuring the blood pressure by stethoscope, left the room during the period of automatic blood pressure recording. The mean blood pressure found during the last forty minutes of each procedure were averaged and the differences between these values tested for significance. Before and after each period of automatic blood pressure recording, the blood pressure was measured simultaneously, by both methods, three times, by placing a stethoscope just below the cuff of the Arteriosonde®. With this procedure only the systolic pressures can be compared,

because after recording the diastolic blood pressure the cuff deflates too rapidly to measure the diastolic blood pressure reliably with the stethoscope.

In 15 normotensive control subjects, (casual blood pressure < 90 mmHg, 4 male and 11 female, ranging in age from 25 to 67 years, mean 38.7 ± 12.8 years) and 19 patients with benign essential hypertension, (casual blood pressure, off treatment > 95 mmHg, 9 male and 10 female, ranging in age from 21 to 58 years, mean 43.4 ± 10.4 years) the blood pressure was measured during 45 minutes of recumbency. In the middle of the first 15 minutes the blood pressure was measured by standard auscultatory manometry and during the last 30 minutes the blood pressure was recorded every 5 minutes with the Arteriosonde^{R}. During the blood pressure recording the subjects and patients were lying supine and left alone. The blood pressure level measured by auscultation in the first 15 minutes was compared with the mean of the blood pressures recorded during the last 30 minutes and with the lowest systolic and diastolic blood pressure recorded during the same period. These lowest blood pressures recorded were considered to represent the basal blood pressure as described by Freis (5), with the modification that in this study the blood pressure was measured automatically in the absence of a nurse or physician.

In 17 normotensive control subjects, 9 male and 8 female, age range 21 - 65 years (39.4 ± 17.1 years) and in 34 patients with benign essential hypertension, 15 male and 19 female, age range 20 - 61 years, (39.8 ± 12.2 years) the blood pressures were measured with the Arteriosonde^{R} during the night (from about 11 p.m. to about 6 a.m.) every half an hour. The mean of the blood pressures recorded during the night and the lowest systolic and diastolic blood pressure recorded during the same period were compared

with the mean of two or three casual blood pressures measured by standard manometry during day-time. All, normotensive subjects and hypertensive patients had about equal intake of sodium and the blood pressure study was performed after they were accomodated to the hospital situation. In all of these 34 hypertensive patients the plasma renin activity (PRA) was measured after 5 days of controlled sodium restriction (sodium intake 15 mEq/24 h) and 3 h of ambulation. The PRA was measured by radioimmunoassay (6).

Non-parametric statistical tests were used to detect differences between paired or unpaired observations (Wilcoxon's test) and to calculate correlation coefficients (Spearman's rank correlation test). The results are expressed as means $\pm$ standard deviation.

RESULTS

Calibration.

From the data of the experiment in which the systolic blood pressure was measured simultaneously by auscultation and with the Arteriosonde^R, a highly significant positive correlation was calculated between both blood pressures (Fig. 1, $r = 0.99$, $n = 60$, $p < 0.001$). The systolic blood pressures measured by auscultation were slightly and not significantly higher than those measured with the Arteriosonde^R; 0.9 ± 0.9 mmHg. In none of the simultaneous measurements the diastolic blood pressure measured by auscultation was higher than that recorded by the Arteriosonde^R.

By comparing the mean of the blood pressures measured

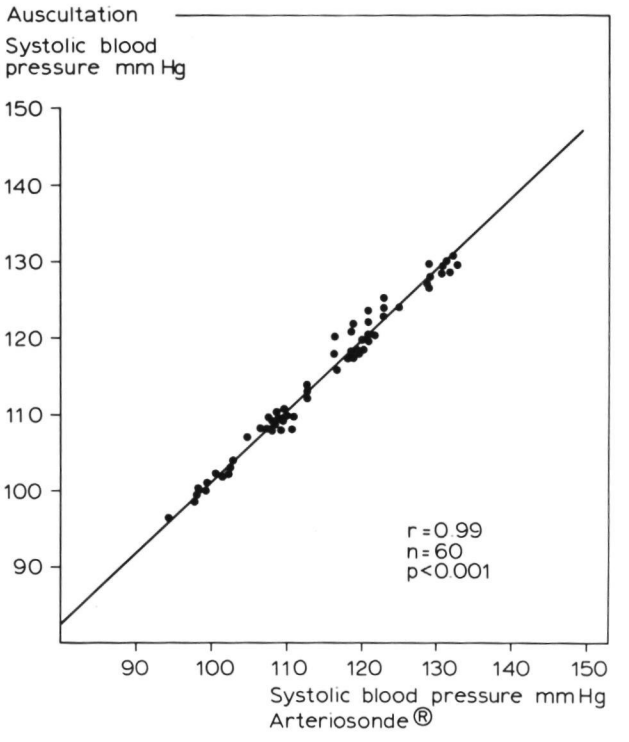

Fig. 1. CORRELATION BETWEEN SYSTOLIC BLOOD PRESSURES MEASURED SIMULTANEOUSLY BY AUSCULTATION AND WITH THE ARTERIOSONDE^{R}.

every 10 minutes during the last 40 minutes of each of 2 successive hours of recumbency by auscultation and with the Arteriosonde^{R}, significantly lower blood pressures, both systolic and diastolic, were recorded with the latter method (Table 1), irrespective of the sequence of the 2 methods. The correlation between the mean of the systolic blood pressures measured by manometry and the mean of the systolic blood pressures recorded with the Arteriosonde^{R} was significantly positive ($r = 0.75$, $p > 0.05$) as was the correlation between both mean diastolic blood pressures ($r = 0.70$, $p < 0.05$).

Table 1. COMPARISON OF BLOOD PRESSURE LEVELS MEASURED IN THE SAME NORMOTENSIVE CONTROL SUBJECTS BY AUSCULTATION AND WITH THE ARTERIOSONDE^{R} DURING THE LAST 40 MINUTES OF EACH OF 2 SUCCESSIVE HOURS OF RECUMBENCY.

	AUSCULTATION		ARTERIOSONDE^{R}
SYSTOLIC BLOOD PRESSURE mmHg	119 ± 11	$p < 0.05$	111 ± 10
DIASTOLIC BLOOD PRESSURE mmHg	76 ± 7	$p < 0.05$	69 ± 9

Comparison of casual blood pressures measured by standard manometry and blood pressures recorded with the Arteriosonde^{R} at day-time.

In 19 hypertensive patients and 15 normotensive control subjects the casual blood pressures measured by auscultation after 5 to 10 minutes of recumbency were compared with the blood pressures recorded during the following 30 minutes, every 5 minutes, with the Arteriosonde^{R}, the patient

Table 2. COMPARISON OF SINGLE CASUAL BLOOD PRESSURES, MEASURED BY AUSCULTATION AFTER 5 TO 10 MINUTES OF RECUMBENCY, WITH THE MEAN OF THE BLOOD PRESSURES RECORDED WITH THE ARTERIOSONDE® DURING 30 MINUTES OF RECUMBENCY WITH 5 MINUTE INTERVALS, AND THE LOWEST BLOOD PRESSURE REACHED IN THAT PERIOD.

	NORMOTENSIVE SUBJECTS n = 15		HYPERTENSIVE PATIENTS n = 19	
	ABSOLUTE VALUE mmHg	RELATIVE DECREASE IN % OF CASUAL READING	ABSOLUTE VALUE mmHg	RELATIVE DECREASE IN % OF CASUAL READING
CASUAL systolic	121 ± 9	-	158 ± 14	-
CASUAL diastolic	76 ± 6	-	104 ± 11	-
ARTERIOSONDE MEAN systolic	111 ± 9	8 ± 6	143 ± 12	8 ± 6
ARTERIOSONDE MEAN diastolic	72 ± 7	7 ± 7	96 ± 9	6 ± 5
ARTERIOSONDE LOWEST systolic	106 ± 9	13 ± 7	135 ± 16	12 ± 6
ARTERIOSONDE LOWEST diastolic	68 ± 8	12 ± 7	91 ± 9	11 ± 6

Table 3. CORRELATION COEFFICIENTS BETWEEN THE SINGLE CASUAL BLOOD PRESSURE READING, THE MEAN OF THE BLOOD PRESSURES RECORDED WITH THE ARTERIOSONDE^{R} DURING 30 MINUTES OF RECUMBENCY AT 5 MINUTE INTERVALS, AND THE LOWEST BLOOD PRESSURES REACHED IN THAT PERIOD.

SYSTOLIC BLOOD PRESSURE RECORDED WITH THE ARTERIOSONDE^{R}

	HYPERTENSIVE PATIENTS n = 19		NORMOTENSIVE SUBJECTS n = 15	
	MEAN	LOWEST	MEAN	LOWEST
CASUAL SYSTOLIC BLOOD PRESSURE	0.67*	0.75*	0.61***	0.63**
ARTERIOSONDE^{R} LOWEST SYSTOLIC BLOOD PRESSURE	0.93*	-	0.92*	-

DIASTOLIC BLOOD PRESSURE RECORDED WITH THE ARTERIOSONDE^{R}

	HYPERTENSIVE PATIENTS n = 19		NORMOTENSIVE SUBJECTS n = 15	
	MEAN	LOWEST	MEAN	LOWEST
CASUAL DIASTOLIC BLOOD PRESSURE	0.72*	0.71*	0.87*	0.77*
ARTERIOSONDE^{R} LOWEST DIASTOLIC BLOOD PRESSURE	0.95*	-	0.97*	-

* $P < 0.001$
** $P < 0.01$
*** $P < 0.05$

being alone in the same silent room.

In table 2 the means of the casual manometer readings, the mean pressures recorded with the Arteriosonde^{R} and the lowest systolic and lowest diastolic blood pressures recorded have been tabulated. Both in the normotensive control subjects and in the hypertensive patients, the mean of the blood pressures recorded with the Arteriosonde^{R} was significantly ($p < 0.01$) lower than the mean of the casual blood pressures. The relative decreases in both, the hypertensive patients and the normotensive control subjects, were about equal.

The correlation coefficients calculated between these blood pressures are shown in table 3. Both in the hypertensive patients and in the normotensive control subjects a significant positive correlation was found between the casual blood pressures and the mean and lowest blood pressures recorded with the Arteriosonde^{R}. The highest correlation coefficients were found between the mean and the lowest blood pressures recorded with the automated device.

Comparison of day-time casual blood pressures measured by standard sphygmomanometry with blood pressures recorded at night with the Arteriosonde^{R}.

In 17 normotensive control subjects and 34 patients with benign essential hypertension data have been obtained according to the protocol described in the methods section. The results are depicted in table 4. Day-time casual blood pressure values are compared with the means of the blood pressure values obtained during the night and with the lowest value reached during this period. The systolic and diastolic blood pressures decreased significantly in both groups during the night ($p < 0.001$). The table also illustrates that the relative decrease to the mean and lowest

Table 4. COMPARISON OF CASUAL BLOOD PRESSURES, MEASURED BY AUSCULTATION AT DAY-TIME, THE MEAN OF THE BLOOD PRESSURES RECORDED WITH THE ARTERIOSONDE^{R} DURING THE NIGHT AT 30 MINUTES INTERVALS, THE LOWEST SYSTOLIC AND DIASTOLIC PRESSURE REACHED IN THAT PERIOD, AND THE VARIABILITY, DEFINED AS THE DIFFERENCE BETWEEN THE CASUAL DAY-TIME AND LOWEST NIGHT-TIME BLOOD PRESSURES IN NORMOTENSIVE CONTROL SUBJECTS AND HYPERTENSIVE PATIENTS.

	NORMOTENSIVE SUBJECTS n = 17		HYPERTENSIVE PATIENTS n = 34	
	ABSOLUTE VALUE mmHg	RELATIVE DECREASE IN % OF CASUAL READING	ABSOLUTE VALUE mmHg	RELATIVE DECREASE IN % OF CASUAL READING
CASUAL systolic	119 ± 12	-	174 ± 26	-
CASUAL diastolic	75 ± 9	-	106 ± 10	-
ARTERIOSONDE MEAN systolic	97 ± 16	18 ± 13	135 ± 29	23 ± 11
ARTERIOSONDE MEAN diastolic	63 ± 10	16 ± 14	91 ± 17	14 ± 12
ARTERIOSONDE LOWEST systolic	81 ± 15	31 ± 11	117 ± 29	33 ± 11
ARTERIOSONDE LOWEST diastolic	49 ± 10	35* ± 12	77 ± 30	26* ± 12
VARIABILITY systolic	37 ± 15	-	57 ± 23	-
VARIABILITY diastolic	27 ± 10	-	29 ± 13	-

* $p < 0.003$

Table 5. CORRELATION COEFFICIENTS BETWEEN THE CASUAL DAY-TIME SYSTOLIC BLOOD PRESSURE, THE LOWEST SYSTOLIC BLOOD PRESSURE RECORDED AT NIGHT, THE VARIABILITY, DEFINED AS THE DIFFERENCE BETWEEN THESE BLOOD PRESSURES, THE AGE AND THE PLASMA RENIN ACTIVITY IN HYPERTENSIVE PATIENTS (UPPER-RIGHT PART) AND NORMOTENSIVE CONTROL SUBJECTS (LOWER-LEFT PART OF THE TABLE).

	SYSTOLIC BLOOD PRESSURE			AGE	PRA
	CASUAL DAY-TIME	LOWEST AT NIGHT	VARIABILITY		
			HYPERTENSIVE PATIENTS n = 34		
CASUAL DAY-TIME		+ 0.69 p < 0.01	+ 0.19 n.s.	+ 0.53 p < 0.01	- 0.05 n.s.
LOWEST AT NIGHT	+ 0.26 n.s.		- 0.43 p < 0.05	+ 0.39 p < 0.05	+ 0.09 n.s.
VARIABILITY	+ 0.52 p < 0.05	- 0.63 p < 0.01		- 0.02 n.s.	- 0.37 p < 0.05
AGE	+ 0.05 n.s.	- 0.36 n.s.	+ 0.28 n.s.		- 0.13 n.s.
	NORMOTENSIVE SUBJECTS n = 17				

Table 6. CORRELATION COEFFICIENTS BETWEEN THE CASUAL DAY-TIME DIASTOLIC BLOOD PRESSURE, THE LOWEST DIASTOLIC BLOOD PRESSURE RECORDED AT NIGHT, THE VARIABILITY DEFINED AS THE DIFFERENCE BETWEEN THESE BLOOD PRESSURES, THE AGE AND THE PLASMA RENIN ACTIVITY IN HYPERTENSIVE PATIENTS (UPPER-RIGHT PART) AND NORMOTENSIVE CONTROL SUBJECTS (LOWER-LEFT PART OF THE TABLE).

	DIASTOLIC BLOOD PRESSURE			AGE	PRA
	CASUAL DAY-TIME	LOWEST AT NIGHT	VARIABILITY		
		HYPERTENSIVE PATIENTS n = 34			
CASUAL DAY-TIME		+ 0.69 p < 0.01	+ 0.11 n.s.	+ 0.33 p < 0.05	- 0.08 n.s.
LOWEST AT NIGHT	+ 0.34 n.s.		- 0.54 p < 0.001	+ 0.14 n.s.	+ 0.14 n.s.
VARIABILITY	+ 0.50 p < 0.05	- 0.66 p < 0.01		+ 0.08 n.s.	+ 0.39 p < 0.05
AGE	+ 0.14 n.s.	+ 0.06 n.s.	- 0.05 n.s.		- 0.13 n.s.
	NORMOTENSIVE CONTROL SUBJECTS n = 17				

systolic and to the mean diastolic blood pressure during the night was not significantly different between the normotensive control subjects and the hypertensive patients. The relative decrease to the lowest diastolic blood pressure was slightly though significantly lower in the hypertensive patients ($p < 0.003$) than in the normotensive controls.

Variability of the blood pressure derived from casual day-time manometry and Arteriosonde^{R} blood pressure recording during the night and its relation to age and plasma renin activity.

The difference between the mean of the blood pressures found at day-time by repeated manometry and the lowest blood pressures recorded with the Arteriosonde^{R} during the night is arbitrarily defined as a measure of the variability of a person's blood pressure. Tables 5 and 6 present the correlation coefficients between the systolic and the diastolic casual blood pressures at day-time, the lowest systolic and diastolic blood pressures during the night, the differences between the respective systolic and diastolic blood pressures, defined as variability, and the age in 17 normotensive subjects and between these blood pressures and the age and the PRA in 34 patients with benign essential hypertension.

From the data of the hypertensive patients in these tables it appears that the higher the casual day-time blood pressures are, the higher is the lowest blood pressure at night. Moreover, the higher the lowest blood pressure values at night are, the lower is the variability. The age of the patients is positively correlated with the height of casual systolic and diastolic blood pressure and with the lowest systolic pressure at night. Interestingly, a loose though significant negative correlation was found between the PRA and the variability. The correlations between

the blood pressure and the PRA, and between the lowest pressure at night and the PRA are not significant.

The casual blood pressures measured in the group of the normotensive control subjects are significantly positively correlated with the variability. As in the hypertensive patients, it appears that the higher the lowest blood pressure values during the night are, the lower is the variability. The age is not significantly correlated with any of the blood pressures measured in the control subjects.

DISCUSSION

Several authors have reported recently the usefulness of prolonged blood pressure recording in the assessment of hypertension and in estimating its variability. In this study a fair correlation was found between the systolic and the diastolic pressures measured by standard manometry and with the automated device Arteriosonde^{R}. In the calibration procedure described, a significant difference between the systolic pressures, obtained simultaneously by manometer readings taken by a physician, and Arteriosonde^{R} recording was not detected. Nevertheless, in the experiment in which a physician took blood pressures during 40 minutes in the room in which the subject was lying supine and in which the Arteriosonde^{R} recorded during another 40 minutes the blood pressure in the identically situated, same normotensive subjects, though in the absence of the physician, the Arteriosonde^{R} recorded systolic and diastolic pressures were significantly lower than those obtained by manometry by a physician. This finding was irrespective of the sequence of both procedures. Concerning the systolic blood pressure, it is clear, from the calibration experiment, that the difference cannot be explained by differences between the sphygmo-

manometer and the Arteriosonde^{R} per se. Regarding the diastolic blood pressure difference, it will be remembered, that in the calibration the diastolic pressure with the conventional manometric procedure was systematically somewhat lower than with the Arteriosonde^{R}, due to a principal difference between both techniques. This finding is in agreement with those of other authors (7, 8). However, in the experiment under discussion, the diastolic pressure measured by the physician with a sphygmomanometer was significantly higher than that recorded with the Arteriosonde^{R}. These considerations lead to the conclusion that the presence of the measuring physician per se caused the higher systolic and diastolic readings with the conventional manometric procedure as was also stated by Irving et al (2). It seems important to be aware that a psychological factor, as detected in this experiment, should be evaluated in studies on the significance of depressor effects on the blood pressure measured. Therefore, in order to get optimal results in experiments centering on blood pressure behaviour, it seems justified to expect automated recording with a well calibrated device to serve such purposes better. In using such devices one has to realize that the blood pressures recorded are indeed significantly lower than with standard manometry. In the experiment presented in this study the mean blood pressures obtained by the Arteriosonde^{R} recording during 30 minutes were about 7 % and the lowest pressures during that period appeared to be about 12 % lower than by casual manometry, both in the normotensive controls as well as in a group of patients with benign essential hypertension.

In the experiment in which the blood pressure was recorded during the night at half hour intervals, earlier reported similar findings were reconfirmed (1). In the group of hypertensive subjects studied a slightly greater

relative resistance to a decrease in the diastolic blood pressure during the night was observed. It is added, that the degree of blood pressure decrease in this experiment with the Arteriosonde^{R} is much more impressive than those reported earlier with invasive procedures (3). Furthermore, blood pressure variability was - though very roughly - approximated and evaluated to some extent. It was calculated that, in hypertensive patients, the variability of the systolic and the diastolic blood pressure does not significantly change with increasing age, whereas the absolute systolic and diastolic casual day-time, as well as the recorded lowest systolic blood pressure with the Arteriosonde^{R} during the night increased the older the patient grew. The correlation between the casual day-time and the night-time lowest pressures - systolic and diastolic - was of the same order of tightness as found in the experiment in which short-term Arteriosonde^{R} recorded blood pressures were compared with casual manometric readings. Remarkably, in the smaller group of normotensive control subjects - in contrast with the hypertensive population - these correlations appeared to be not significant. A significant difference in the variances of the mean values between these groups did not exist. The lowest night-time blood pressures were significantly negatively correlated with the variability in both the hypertensive patients and the normotensive subjects. However, the casual day-time pressures appeared to be significantly and positively correlated with the variability in the normotensive subjects, not significantly however in the hypertensive patients. A significant difference in the standard deviation of the respective values of the variability of the blood pressure, as defined in this study, was not detectable. These considerations taken together, the conclusion seems inevitable that the casual systolic and diastolic day-time blood pressure levels in normotensive

subjects are per se much more varying qualities than those in hypertensive patients.

The interesting observation that the variability of both the systolic and the diastolic blood pressure was - though loosely - significantly and negatively correlated with the renin state of the hypertensive patient deserves some comment. There is an current discussion and dispute in the literature about the meaning of the concept 'low renin' hypertension. In this laboratory by 'low renin' hypertension is meant a hypertensive disorder with a renin activity, measured after stimulation by sodium restriction for 5 days and 3 h of ambulation lower than in normotensive controls. In earlier studies in this laboratory it has been shown that a classification according to that procedure differs from a classification based upon renin measurement without rigid sodium restriction (9) or based upon renin measurements after stimulation with furosemide (10). The negative correlation found between the variability of the blood pressure and the in this way determined renin state in hypertensive patients means, that a greater variability of pressure concurs with a lower plasma renin activity. This provocative statement necessitates additional studies. Finally, in this limited number of hypertensive patients the negative correlation between plasma renin activity and the age of the patient was not found to be significant, in contrast with the results of other investigators (11 - 13) and the observations in a larger series of patients in this laboratory as well (14).

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Chapter V

A WITHIN PATIENT COMPARISON OF CHLORTHALIDONE, SPIRONOLACTONE AND PROPRANOLOL TREATMENT IN NORMORENINEMIC ESSENTIAL HYPERTENSION.

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ABSTRACT

In this study the blood pressure lowering effects of long-term treatment with the diuretic chlorthalidone, the anti-aldosterone drug spironolactone and the β-blocker propranolol have been compared in the same patients. Besides the effect of the drugs on blood pressure, plasma renin activity and plasma aldosterone, attention has been paid to changes in body weight, plasma electrolyte concentrations and glomerular filtration rate. All patients had mild essential hypertension with normal plasma renin activity.

All three drugs decreased the blood pressure significantly and none of the agents was superior in their blood pressure lowering effect. The blood pressure did not normalize. The data suggest that neither volume factors, relative hyperactivity of the renin-aldosterone system nor β-adrenergic hyperactivity is the prime mover in normoreninemic hypertension. Long-term chlorthalidone treatment resulted in slight hyperreninism with concomitant changes in plasma aldosterone. The body weight decreased significantly. The concentrations of sodium, potassium and chloride in plasma decreased. The creatinine clearance was unimpaired. Chronic treatment with spironolactone resulted in more marked hyperreninism and high plasma aldosterone concentrations were found. The body weight decreased significantly. The concentrations of plasma sodium and chloride were lower than without treatment and a significant hyperkalemia occurred. The glomerular filtration rate decreased. Long-term propranolol treatment resulted in marked suppression of the plasma renin activity and plasma aldosterone levels. The body weight increased significantly. The concentrations of plasma sodium and chloride decreased significantly and a slight though significant hyperkalemia

occurred.The glomerular filtration rate decreased.

Hyperreninemia (by spironolactone and chlorthalidone), effective hyperaldosteronism (by chlorthalidone) and volume retention (by propranolol) are considered to represent expressions of mechanisms counteracting the depressor effects of these different pharmacological maneuvers, leading to the maintenance of a supranormal blood pressure.

INTRODUCTION

There is good evidence that lowering mild elevated blood pressures prevents later rises (1 - 4). In recent years the use of diuretics and β-blockade has increased considerably to that purpose.

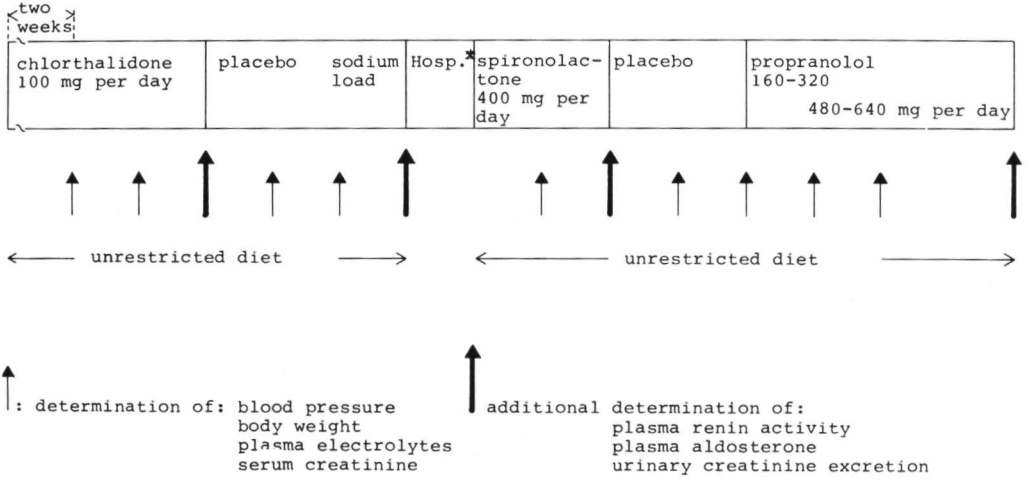
Comparison of the effectiveness of diuretics and β-blockade in the same hypertensive patients is the subject of this study. In order to exclude bias of the results by including 'low renin' or 'high renin' patients, normorenin-emics have been deliberately chosen. To relate the depressor effect of diuretics and β-blockade to the activity of the renin-angiotensin-aldosterone system during treatment more delicately, the thiazide-type diuretic agent chlortha-lidone and the anti-aldosterone agent spironolactone have been included in the comparison with β-blockade by propra-nolol. Besides the activity of the renin-angiotensin-aldosterone system and the depressor effect, changes in body weight, glomerular filtration rate and plasma electro-lytes have been given attention.

Surprisingly, similar though not normalizing blood pressure lowering by these three principally pharmacologi-cally different agents was observed in the presence of marked differences in a number of seemingly related variables.

METHODS

Patients with mild hypertension were selected at random from the outpatient clinic. Their diastolic blood pressure measured, off treatment, by auscultation, after ten minutes of recumbency was higher than 95 mmHg. Secondary causes of hypertension, such as pheochromocytoma, Cushing's

syndrome, renal artery stenosis and primary aldosteronism were exluded by appropriate laboratory and roentgenographic procedures. The plasma renin activity (PRA) was measured after 5 days of controlled sodium restriction as well as after 5 days of chlorthalidone treatment. After both regimens blood for PRA was taken at noon after 3 h of ambulation. With both procedures patients with low and normal PRA can be discriminated (5). Eleven patients, 5 female and 6 male, with a PRA in the range of normotensive controls were studied. Their age ranged from 32 to 59 (mean age 44 years). Cardiac and renal function were unimpaired in all of them. After giving their informed consent the patients were treated according to the protocol as depicted in Fig. 1.



* Hospitalization

Fig. 1. Study design.

The patients were subsequently treated with chlorthalidone, 100 mg per day for at least 6 weeks, spironolactone, 400 mg per day during 4 weeks and propranolol during 8 weeks in increasing doses up to 640 mg per day in 9 patients, and 320 mg per day in two patients. The highest doses of propranolol were administered for at least 5 weeks. During the study period the patients were seen at 2 week intervals. Each visit the blood pressures were recorded between 8 and 10 a.m. with an automated device (Arteriosonde^{R}) every 5 minutes during 40 minutes of recumbency. The average of the pressures recorded during the last 30 minutes was considered as the representative blood pressure of the visit. The patients were weighed and blood was taken for the measurements of the concentrations of sodium, potassium and chloride in plasma and for the measurement of the creatinine concentration in serum. At the end of each treatment period the PRA and the concentration of plasma aldosterone were measured in blood taken after 3 h of ambulation at noon and the creatinine excretion was measured in urine collected during the preceeding 12 hours.

The treatment periods were separated by a placebo phase. In the 5th week of the first placebo phase the basal measurements of the blood pressure, electrolyte concentrations in plasma and creatinine concentration in serum were done. In the 6th week of this period the patients consumed an extra sodium load (9 grams of NaCl extra per day) for 5 days, on the last day of which the same measurements were performed as at the end of each treatment schedule. A short clinical observation followed, during which the PRA was measured after 5 days of controlled sodium restriction (15 mEq of sodium per day) and 3 h of ambulation at noon and the aldosterone secretory rate was measured on the 6th day of sodium restriction during 24 h of recumbency. The potassium intake during hospitalization ranged from 50 - 90 mEq per

day. The creatinine clearance was measured repeatedly during hospitalization. After the fourth week of the second placebo period the same observations were done as in the fifth week of the first placebo period. In the intervals between measurements each subject carried on a normal out-patient life without restriction of dietary sodium intake. The sodium, potassium and chloride concentrations in plasma and the creatinine concentration in serum and urine were measured by standard laboratory procedures. The PRA was measured by radioimmunoassay. The PRA measured in 22 normotensive control subjects (17 - 55 yr) after 3 h of ambulation at noon without any dietary regimen was 4.6 $\pm$ 0.5 $ng.ml^{-1}.3\ h^{-1}$ (6). PRA values measured after clinical sodium restriction for 5 days and 3 h of ambulation at noon being higher than 11.5 $ng.ml^{-1}.3\ h^{-1}$ were considered as normal in our laboratory (5). The PRA measured after 5 days of chlorthalidone treatment in an in age comparable group of 11 (5 female and 6 male) normotensive control subjects was 40.7 $\pm$ 5.6 $ng.ml^{-1}.3\ h^{-1}$. The plasma aldosterone concentration and the aldosterone secretory rate were measured by radioimmunoassay. The aldosterone secretory rate during sodium restriction measured in an in age matched group of normotensive control subjects was 415 $\pm$ 49 γ/24h. Non-parametric statistical methods were used to detect differences between paired observations (Wilcoxon's test). The results are expressed as means $\pm$ SE.

Observations during the placebo periods.

The blood pressures recorded automatically by the Arteriosonde[R] in these patients in the first placebo period were compared with those measured in an in age and sex ratio comparable group of normotensive control subjects. The systolic and the diastolic blood pressure as well as the calculated mean arterial pressure in the former group was significantly higher than the respective blood pressures recorded in the same way in the latter group (Table 1).

Table 1. COMPARISON OF SYSTOLIC (P SYST) AND DIASTOLIC (P DIAST) BLOOD PRESSURE AND THE CALCULATED MEAN ARTERIAL PRESSURE (MAP) I NORMOTENSIVE CONTROL SUBJECTS AND HYPERTENSIVE PATIENTS. THE DATA IN THE FORMER GROUP WERE OBTAINED IN A WAY IDENTICAL TO THAT DESCRIBED FOR THE LATTER IN THE METHODS SECTION.

	NORMOTENSIVE CONTROL SUBJECTS n = 11	P	HYPERTENSIVE PATIENTS n = 11
P syst	111 ± 2	< .001	152 ± 5
P diast	72 ± 2	< .001	99 ± 2
MAP	85 ± 2	< .001	116 ± 2

mmHg

Compared to the measurements done in normotensive control subjects, the 11 hypertensive patients had a normal PRA after sodium restriction ranging from 12.6 to 38.1

$ng.ml^{-1}.3\ h^{-1}$ (mean 20.9 ± 2.7 $ng.ml^{-1}.3\ h^{-1}$). After 5 days of chlorthalidone treatment the mean PRA was not significantly different from the mean found in an in age and sex ratio comparable group of normotensive control subjects (48.0 ± 6.2 and 40.7 ± 5.6 $ng.ml^{-1}.3\ h^{-1}$ respectively, ns). The aldosterone secretory rate measured after sodium restriction was significantly lower than the value found in age matched normotensive control subjects (265 ± 40 and 415 ± 49 γ/24h respectively, $p < 0.01$). Sodium loading with 9 grams of sodium chloride per day in addition to the unrestricted diet resulted in a suppression of the PRA to 3.0 ± 0.6 $ng.ml^{-1}.3\ h^{-1}$ whereas the plasma aldosterone concentration at the same time was 6.7 ± 1.3 pg/100 ml. The creatinine clearance measured after sodium loading was not significantly different from the mean creatinine clearance measured during hospitalization (117 ± 5 and 120 ± 5 ml/min respectively, ns). Sodium loading did not result in a significant rise in body weight in these patients (69.5 ± 2.6 before and 69.7 ± 2.6 kg at the end of 5 days of sodium loading) and symptoms nor signs of heart failure were found.

To study the effect of the different anti-hypertensive agents, the observations done in the fifth week of the first placebo period were compared to those obtained at the end of each treatment schedule. The results obtained in the first placebo period did not differ significantly from those obtained at the end of the second placebo period.

Observations during treatment.

Out-patient observations after chlorthalidone treatment.

The results of anti-hypertensive treatment with chlorthalidone therapy for 6 weeks (100 mg per day) were compared with the results measured in the fifth week of the first placebo period (Table 2).

Table 2. COMPARISON OF CLINICAL AND BIOCHEMICAL VARIABLES MEASURED AFTER LONG-TERM CHLORTHALIDONE TREATMENT WITH THOSE MEASURED AFTER THE PLACEBO PERIOD.

	PLACEBO	P	CHLORTHALIDONE
P syst	152 ± 5	< .005	130 ± 4
P diast	99 ± 2	< .032	93 ± 3
MAP mmHg	116 ± 2	< .006	105 ± 3
BODY WEIGHT KG	69.5 ± 2.6	< .005	67.7 ± 2.0
PLASMA SODIUM	143 ± 1	< .029	141 ± 1
PLASMA POTASSIUM	3.9 ± 0.1	< .004	3.2 ± 0.1
PLASMA CHLORIDE $mEq.l^{-1}$	107 ± 1	< .003	99 ± 1
SERUM CREATININE $\mu mol.l^{-1}$	87 ± 6	n.s.	86 ± 6
CREATININE CLEARANCE $ml.min^{-1}$	120 ± 5*	n.s.	117 ± 6

* measured during hospitalization
n.s.: not significant.

The systolic and the diastolic blood pressure, measured with the Arteriosonde^{R} and the calculated mean arterial pressure decreased significantly. The mean body weight was significantly lower, 1.8 kg, than in the placebo period. The concentrations of sodium, potassium and chloride decreased significantly. The serum creatinine concentration measured after long-term chlorthalidone treatment did not differ significantly from the value found in the placebo phase. The creatinine clearance calculated was not significantly different from the mean creatinine clearance measured during hospitalization or after sodium loading.

Out-patient observations after spironolactone treatment.

The results of anti-hypertensive treatment with spironolactone therapy for 4 weeks (400 mg per day) were compared with the results measured in the placebo period (table 3).

The systolic and diastolic blood pressure recorded and the calculated mean arterial pressure decreased significantly during spironolactone treatment. The mean body weight measured after spironolactone was significantly lower, 1.9 kg, than in the placebo period. The concentrations of sodium and chloride decreased significantly and the plasma potassium concentration increased significantly. The serum creatinine concentration measured after chronic spironolactone treatment was significantly higher than the value found in the placebo period. Accordingly, the creatinine clearance calculated after spironolactone was significantly lower than the mean creatinine clearance measured during hospitalization or after sodium loading.

Out-patient observations after propranolol treatment.

The results of antihypertensive treatment with propranolol treatment for 8 weeks (up to 640 and 320 mg per

Table 3. COMPARISON OF CLINICAL AND BIOCHEMICAL VARIABLES MEASURED AFTER LONG-TERM SPIRONOLACTONE TREATMENT WITH THOSE MEASURED AFTER THE PLACEBO PERIOD.

	PLACEBO		P	SPIRONOLACTONE	
P syst	152	± 5	<.003	132	± 5
P diast	99	± 2	<.046	94	± 2
MAP mmHg	116	± 2	<.007	106	± 3
BODY WEIGHT KG	69.5	± 2.6	<.004	67.6	± 2.2
PLASMA SODIUM	143	± 1	<.008	139	± 1
PLASMA POTASSIUM	3.9	± 0.1	<.014	4.4	± 0.1
PLASMA CHLORIDE $mEq.l^{-1}$	107	± 1	<.004	102	± 1
SERUM CREATININE $\mu mol.l^{-1}$	87	± 6	<.014	99	± 5
CREATININE CLEARANCE $ml.min^{-1}$	120	± 5[x]	<.004	93	± 3

x measured during hospitalization

Table 4. COMPARISON OF CLINICAL AND BIOCHEMICAL VARIABLES MEASURED AFTER LONG-TERM PROPRANOLOL TREATMENT WITH THOSE MEASURED AFTER THE PLACEBO PERIOD.

	PLACEBO		P	PROPRANOLOL	
P syst	152	± 5	<.006	133	± 5
P diast	99	± 2	<.019	90	± 3
MAP mmHg	116	± 2	<.003	105	± 4
BODY WEIGHT KG	69.5	± 2.6	<.013	71.8	± 2.3
PLASMA SODIUM	143	± 1	<.017	140	± 1
PLASMA POTASSIUM	3.9	± 0.1	<.049	4.2	± 0.1
PLASMA CHLORIDE $mEq.l^{-1}$	107	± 1	<.036	104	± 1
SERUM CREATININE $\mu mol.l^{-1}$	87	± 6	<.039	94	± 6
CREATININE CLEARANCE $ml.min^{-1}$	120	± 5*	<.008	99	± 5

* measured during hospitalization

day, at least during the last 5 weeks) were also compared with the observations done in the first placebo period (table 4). Propranolol treatment caused a significant decrease in the systolic, the diastolic and the mean arterial pressure. The mean body weight however increased significantly during propranolol treatment, 2.3 kg. The concentrations of sodium and chloride decreased significantly during propranolol medication, the plasma potassium concentration however was significantly higher after propranolol. The serum creatinine concentration measured after long-term propranolol treatment was significantly higher than the value found in the placebo period. The creatinine clearance after propranolol was significantly lower than the mean creatinine clearance measured during hospitalization or after sodium loading.

Comparison of the results after chlorthalidone, spironolactone or propranolol.

The results of treatment of the same patients with either the thiazide-type diuretic chlorthalidone, or the aldosterone-antagonist spironolactone, or the ß-blocking agent propranolol are compared in table 5.

The blood pressure levels reached with each drug appeared to be equal. The small differences found between the mean systolic blood pressures measured after the three treatment schedules were not significant, neither were the differences found between the mean diastolic pressures nor between the calculated mean arterial pressures.

The mean body weight measured at the end of propranolol treatment was significantly higher than the body weights measured after chlorthalidone or spironolactone. The mean body weight measured after chlorthalidone was not significantly different from that after the latter.

Table 5. COMPARISON OF THE EFFECTS OF LONG-TERM ANTIHYPERTENSIVE TREATMENT WITH CHLORTHALIDONE, SPIRONOLACTONE AND PROPRANOLOL IN ELEVEN PATIENTS WITH NORMORENINEMIC ESSENTIAL HYPERTENSION.

	CHLORTHALIDONE	P	SPIRONOLACTONE	P	PROPRANOLOL	P#
P syst	130 ± 4	n.s.	132 ± 5	n.s.	133 ± 5	n.s.
P diast	93 ± 3	n.s.	94 ± 2	n.s.	90 ± 3	n.s.
MAP mmHg	105 ± 3	n.s.	106 ± 3	n.s.	105 ± 4	n.s.
BODY WEIGHT KG	67.7 ± 2.0	n.s.	67.6 ± 2.2	<.006	71.8 ± 2.3	<.008
PLASMA SODIUM	141 ± 1	<.044	139 ± 1	n.s.	140 ± 1	n.s.
PLASMA POTASSIUM	3.2 ± 0.1	<.004	4.4 ± 0.1	n.s.	4.2 ± 0.1	<.004
PLASMA CHLORIDE $mEq.l^{-1}$	99 ± 1	<.004	102 ± 1	n.s.	104 ± 1	<.003
SERUM CREATININE $\mu mol.l^{-1}$	86 ± 6	<.005	99 ± 5	n.s.	94 ± 6	<.014
CREATININE CLEARANCE $ml.min^{-1}$	117 ± 6	<.005	93 ± 3	n.s.	99 ± 6	<.009
PLASMA RENIN ACTIVITY $ng.ml^{-1}.3h^{-1}$	26.3 ± 4.9	n.s.	47.0 ± 14.3	<.006	1.8 ± 0.2	<.006
PLASMA ALDOSTERONE $pg.100ml^{-1}$	23.0 ± 3.2	<.001	61.9 ± 11.8	<.001	8.9 ± 1.3	<.001

p values versus chlorthalidone
n.s.: not significant.

A small though significant difference was found between the concentrations of sodium in plasma after chlorthalidone and spironolactone. The lower plasma sodium concentration was measured after spironolactone. The mean sodium concentration after propranolol was in between those after chlorthalidone and spironolactone and did not differ significantly from both. The mean plasma potassium concentration after chlorthalidone was significantly lower than the mean plasma potassium concentration after spironolactone or propranolol. The mean plasma potassium concentration measured after spironolactone was not significantly different from the mean plasma potassium concentration after propranolol. The concentrations of chloride in plasma were about equal after spironolactone and propranolol, but both significantly higher than the plasma chloride concentration measured after chlorthalidone treatment. The creatinine concentration in serum and the calculated creatinine clearance were about the same after both spironolactone and propranolol treatment. The serum creatinine concentration measured after chlorthalidone however was significantly lower and the creatinine clearance significantly higher than after spironolactone or propranolol. The PRA after 4 weeks of spironolactone was higher than after chlorthalidone. However, this difference appeared to be not significant due to the large spread of values measured after 4 weeks of spironolactone. The PRA-values measured after propranolol were extremely low. The plasma aldosterone concentration measured after chronic chlorthalidone treatment was significantly lower than the plasma aldosterone concentration after long-term spironolactone but both were significantly higher than the plasma aldosterone concentration measured after propranolol.

The antihypertensive potential of diuretics, both of the thiazide-type as well as of the anti-aldosterone-type, and of β-adrenergic blocking drugs have been reported in numerous studies. However, to the best of our knowledge no study has been published in which the antihypertensive effects of the diuretics chlorthalidone and spironolactone, and the effect of the β-blocker propranolol have been compared in the same hypertensive individuals. Normoreninemic patients have been chosen deliberately to exclude bias of the results by studying patients of possible subgroups of hypertensives such as the 'low-renin' hypertensives which according to some investigators might hyperreact to an anti-aldosterone agent (7, 8, 9) or the 'high-renin' hypertensive patients according to others probably more sensitive to the depressor effect of β-blocking drugs (10). The patients studies were, according to clinical criteria, of the benign essential hypertension type and did secrete amounts of aldosterone, after rigid sodium restriction, even lower than in normotensive controls.

By using the non-parametric Wilcoxon's test for paired observations the significant decrease of the mean blood pressure by each of the drug schedules can be considered to be representative for each patient and therefore the probability is exceedingly low that excessive depressor effects in individual patients might be responsible for the observed depressor effects of these drugs. Indeed, the data of the blood pressure changes in each individual patient were in accordance with this thesis.

Remarkably, the mean decrease of the blood pressure level (systolic, diastolic and mean arterial pressure) with either of these three drugs was about equal. Furthermore, none of these three drugs given in fairly high doses

normalized the mean blood pressure in this sample of mild hypertensives. This equal though not normalizing, decrease of the blood pressure by these three drugs suggests that volume factors neither hyperactivity of the hormonal renin-angiotensin-aldosterone system nor the ß-adrenergic system play the predominant role in the etiology of normoreninemic benign hypertension.

Interestingly, 6 weeks of chlorthalidone and 4 weeks of spironolactone treatment decreased body weight almost identically. On the contrary, propranolol treatment induced a significant weight gain. In two of the 11 patients of this study clinically detectable ankle edema was observed. By virtue of the same statistical arguments as given above, it is highly improbable that these weight decreases and increases have been caused by excessive weight changes in some individuals. Obvious changes in caloric intake have not been admitted by these patients during either treatment. Therefore it is considered that the weight changes represent changes in extracellular fluid volume. In fact Sederberg-Olsen (11) et al found a significant rise in extracellular fluid volume during long-term treatment with propranolol. Taken this into account, an almost identical blood pressure lowering occurred in this group of normoreninemic hypertensives both in the presence of volume depletion either by chlorthalidone or spironolactone as well as in the presence of supposed volume surfeit by propranolol.

Most authors agreed that the depressor activity of diuretics is initiated by hypovolemia. Concerning their chronic antihypertensive effect other hemodynamic adjustments have not been excluded (12). Hypovolemia was measured to exist even after treatment for several weeks or months (13 - 15). Indirect evidence for persisting hypovolemia has been given by Vaughan et al (16) who found sustained elevations of plasma renin activity and aldosterone excretion

during long-term treatment with either chlorthalidone or spironolactone. The depressor activity of propranolol was accompanied by signs and symptoms of decreased cardiac performance; the pulse rate decreased significantly (from 79 ± 2 per minute before to 64 ± 3 after propranolol) in the presence of blood pressure lowering and clinical sings of volume retention - increase in body weight and ankle edema in some of the patients - occurred. It is added that in this group of patients latent cardiac failure was not present as was proven by the lack of volume retention during sodium loading with extra NaCl for 5 days. In a number of studies it has indeed been indicated that β-blockade depresses the cardiac index (17, 18). It is amply known that decrease of cardiac performance leads to sodium and water retention by the kidneys (19, 20) and ensuing weight gain. Several investigators, however, did not observe signs and symptoms of volume retention during β-blockade in patients without cardiac failure (17, 21). In addition to the decrease in cardiac performance, β-blockade appeared to suppress the action of the renin-angiotensin-aldosterone system, as was earlier reported by other investigators (10, 18, 21, 22). In this study the renin and aldosterone levels after propranolol were as low as those measured after sodium loading. This suppression might contribute to the blood pressure lowering effect of β-blockade. However, other β-blocking drugs have been reported to decrease the blood pressure in the absence of a discernable decrease in plasma renin activity (23, 24). Therefore one might indeed doubt if the direct action of β-blockade on renin release and the consequently occurring hypoaldosteronism were the main factor causing a decrease in blood pressure. From the overall evidences of this study the decrease in cardiac performance is considered to be the more important factor in blood pressure control in

this group of patients. It should be mentioned however, that central nervous system actions of this pharmacological maneuver cannot be excluded to contribute to the blood pressure lowering effect of β-blockade.

By comparing the effects observed during chlorthalidone, spironolactone and propranolol treatment it is interesting to delineate the changes in the activity of the renin-angiotensin-aldosterone system and those in plasma electrolytes and renal function. In the presence of almost identical weight decreases by volume depletion and indistinguishable depressor actions of chlorthalidone and spironolactone, the plasma aldosterone levels after the latter appeared to be significantly higher than after the former, whereas the plasma renin activities were not significantly different. It is quite conceivable that the significantly higher plasma potassium concentration after spironolactone treatment was responsible for this difference between the plasma aldosterone levels. In favor of this explanation a significant positive correlation between plasma potassium and plasma aldosterone levels was calculated during spironolactone treatment (Spearman's rank-correlation test; $r = 0.66$, $n = 11$, $p < 0.05$). Still in another aspect the thiazide-type of diuretic action differed from the anti-aldosterone treatment. After both diuretic schedules the mean plasma sodium concentration decreased, however the mean concentration of sodium after spironolactone was significantly lower than after chlorthalidone. Accordingly, plasma chloride levels after both treatment decreased, however after spironolactone the mean chloride level was significantly higher than after chlorthalidone. As mentioned already, a significant hypokalaemia persisted after 6 weeks of chlorthalidone whereas the mean potassium concentration after 4 weeks of spironolactone was significantly higher than in the placebo period. These differences in the effects of chlorthalidone

and spironolactone on plasma sodium, chloride and potassium concentrations could be expected from the main anti-aldosterone effect of spironolactone and the pure natriuretic, chloruretic and consequent - due to secondary aldosteronism - kaliuretic effect, of chlorthalidone. Concerning the changes in the concentrations of these plasma electrolytes after chronic β-blockade, it is remembered that the plasma sodium and chloride concentrations were significantly lower and the plasma potassium concentration significantly higher than in the placebo period. These changes after propranolol treatment were comparable and not significantly different from the corresponding changes after spironolactone. It is therefore tempting to speculate that the observed suppressed activity of the renin-angiotensin-aldosterone system was responsible for these propranolol induced electrolyte changes as was the anti-aldosterone action in case of spironolactone.

After both spironolactone and propranolol treatment the calculated creatinine clearance was significantly lower than after chlorthalidone. After the latter the creatinine clearance did not differ significantly from that in the placebo period. The decrease of the glomerular filtration rate after propranolol is in accordance with an earlier report by Ibsen et al (26) and these authors stated that this change could be due to the decrease in cardiac performance. Concerning the decrease of the glomerular filtration rate after spironolactone one might wonder if the anti-aldosterone activity of the drug were responsible for this effect. Indeed, it is well known that in clinical situations of hypoaldosteronism, e.g. in adrenal insufficiency, or in patients with isolated hypoaldosteronism decreased glomerular filtration rate is not uncommon (26 - 28). It remains to be explained why after chronic chlorthalidone treatment, in the presence of volume depletion, the creatinine clearance does not differ from that in the control period.

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SUMMARY

The activity of the renin-angiotensin system is considered to represent a marker of a complex of factors that can cooperate in the pathogenesis of hypertension and that might have prognostic significance with regard to its natural course and to the effect of differing modes of treatment. In extreme situations, e.g. advanced or malignant hypertension and primary mineralocorticoidism, the renin activity in most cases is extremely high or low. In recent years a discussion has been going on in the literature with regard to the significance of the classification of hypertensive patients in low, normal and high renin individuals. A classification of this kind requires a careful definition of what is normal.

It is well known that the plasma renin activity in normotensive controls varies widely from subject to subject even under rigidly controlled conditions in a metabolic ward. Besides, intra-individually the renin activity shows spikes and nadirs not yet understood. In this context it seems unnecessary to comment that standardization of methods to measure renin is urgently needed. Furthermore, attention should be paid to the way in which the renin status of a hypertensive individual can be screened reliably even on an out-patient base. In this thesis some aspects of the renin-angiotensin system in essential hypertension are presented.

In chapter I, the reliability of the measurement of plasma renin activity by radioimmunoassay is described. The incubation and elution procedures used in this radioimmunoassay were the same as those in the formerly used bioassay. The amount of angiotensin I generated during the first three hours of incubation appeared to be linear with time. Only a negligible amount of angiotensin I was found in non-

incubated plasma samples as well as in plasma samples incubated for 3 hours at 4° C. Measurable amounts of angiotensin I could not be detected in plasma samples from nephrectomized patients with this radioimmunoassay procedure. The interassay variation was about 13 %. The recovery of the assay was 89 %. The lowest measurable amount of angiotensin I in the incubation mixture was 5 pg. From this value the lowest detectable PRA value was calculated to be 0.5 $ng.ml^{-1}.3\ h^{-1}$.

The specificity of the method was illustrated by the comparison of the assay results with those of the formerly used bioassay. Using the same incubation and extraction procedures a highly significant positive correlation was found between the results of both methods. The radioimmunoassay values were 2.4 $\pm$ 0.6 times higher than the bioassay results. This difference between the results of both assays was to be expected because of the different standards of angiotensin used. In the radioimmunoassay angiotensin I and in the bioassay angiotensin II was used as the standard.

To arrive at a better understanding of the widely differing ranges of renin activities reported to occur in normotensive control subjects, two observations in this study seem important. First, the response differences found between the antibody and the different angiotensin I standards tested and secondly, the difference found between the results of two radioimmunoassay techniques, one with and one without extraction of angiotensin I from the incubation mixture. To enable comparison of assay results in different laboratories it seems important to express the plasma renin activities in terms of an international standard renin. The radioimmunoassay used in this laboratory was calibrated to an internationally accepted standard renin and after recalculation of the results obtained in normotensive control subjects the range of values was in much better agreement

with those reported by other laboratories using the same standardization.

In Chapter II results of the measurement of plasma renin activity after different renin stimulating maneuvers are described. In the introduction section of this chapter it is emphasized that the wide range in the reported frequency of so called 'low renin' hypertension can be partly due to the widely varying renin stimulating maneuvers used. The measurement of the plasma renin activity after 5 days of rigidly controlled sodium restriction in hospital and 3 h of ambulation has been considered to be the preferred method in a number of studies. With this sodium restriction test as the reference method, two out-patient renin stimulating methods have been compared with regard to their discriminating capacity to classify hypertensive patients as low- or normal renin patients. The use of furosemide (140 mg, orally in 3 doses in 18 h) in out-patients did not result in the detection of the same low renin hypertensives as the sodium restriction test. The interpersonal variability in the reponse to high doses of furosemide given in a very short time was thought to account for this discrepancy. When chlorthalidone was given (100 mg per day for 5 days) to out-patients, however, the same patients were detected to be of the low renin type of hypertension as those selected with the in-patient sodium restriction test. Moreover, a highly significant positive correlation was calculated between the results of both tests.

In the first part of Chapter III additional data are given about the comparison of both tests. The renin values found after chlorthalidone and those found after sodium restriction in normotensive control subjects are reported. Only two patients with borderline renin values after sodium-restriction were misclassified. The correlation between the results of both tests in these 60 patients was highly

significantly positive ($r = 0.77$, $p < 0.001$).

In the second part of Chapter III the results of the classification with the chlorthalidone test are compared with those after different levels of sodium intake. From these data it is clear that by renin measurements without stimulation of renin secretion a radically different classification in lower and higher renin levels would be obtained than with the chlorthalidone test. Moreover, most renin values measured in the hypertensive patients after moderate, unrestricted or high sodium intake were within the range found in normotensive control subjects on unrestricted sodium intake.

The data in Chapters II and III clearly illustrate that, in order to be able to classify on an out-patient base the same group of patients as hypo- or normoreninemics as with the sodium restriction procedure in hospital. The different results obtained by tests in which renin secretion was not stimulated, or stimulated with considerable interpersonal variation with reference to the sodium restriction procedure might explain, to some extent, the current dispute about etiology, effectiveness of pharmacologically different antihypertensive drugs and prognosis in low renin and normal renin hypertension.

With the tools described in the previous chapters of this thesis, patients with low and normal renin activity were selected from the out-patient clinic to cooperate in a study covering the effectiveness of different therapeutic approaches in relationship to their renin state. Special attention has been given to the measurement of the blood pressure in this study.

The automated device and its usefulness in the blood pressure recording is described in Chapter IV. From the first part of the study it appeared that the blood pressure levels measured simultaneously by auscultation and by

automatic recording did not differ significantly. Furthermore, blood pressure measurements by auscultation (with a physician in the room of the patient) and by automated recording (in the absence of the physician) during successive periods of 40 minutes were compared. With the former method significantly higher blood pressures were obtained than with the latter. Compared with casual blood pressure readings significantly lower blood pressures were obtained during 30 minutes of automatic recording at day-time. Both in normotensive control subjects and in hypertensive patients the mean of the systolic and diastolic blood pressure was about 7 % and the lowest value about 12 % below the casual reading. During the night a more pronounced blood pressure decrease was observed. In normotensive control subjects and hypertensive patients the lowest systolic pressure recorded during the night was about 32 % lower than the casual systolic blood pressure. With regard to the diastolic blood pressure, the relative difference between the casual reading and the lowest diastolic blood pressure recorded during the night was 35 % in normotensive control subjects and 26 % in hypertensive patients ($p < 0.003$).

The absolute difference between the casual blood pressure measured at day-time and the lowest blood pressure recorded during the night was defined as the variability of the blood pressure. The height of the variability was not related to age either in hypertensive patients or in normotensive control subjects. In the hypertensive patients a significant negative correlation was calculated between the variability and the plasma renin activity. The height of the day-time or night-time blood pressure was not significantly correlated with the height of the plasma renin activity. From the correlation coefficients calculated between the casual blood pressure, the lowest blood pressure during the night and the variability in normotensive control

subjects and hypertensive patients the conclusion was drawn that in normotensive control subjects the casual day-time blood pressure is a more varying quality than in hypertensive patients.

In Chapter V the first part of the above mentioned assessment of the relationship between the renin state of the patient and its significance in the choice of treatment of uncomplicated hypertension is described. In this study three antihypertensive maneuvers in the same group of normoreninemic hypertensive patients have been compared. The antihypertensive drugs used were the saluretic drug chlorthalidone, the anti-aldosterone agent spironolactone and the β-adrenergic blocking drug propranolol. The first drug was included in this protocol because of its reported beneficial effect when used as basic maneuver in treating hypertensive patients. In order to study the role of the renin - angiotensin - aldosterone system in essential hypertension more carefully the effects of the aldosterone antagonist spironolactone and the renin inhibitor propranolol have been compared with the effect of saluretic treatment with chlorthalidone. It should be mentioned, however, that other factors contribute to the blood pressure decrease obtained with these drugs.

In addition to the effects of the drugs on blood pressure, plasma renin activity and plasma aldosterone, attention has been paid to changes in body weight, plasma electrolyte concentrations and glomerular filtration rate.

Interestingly, with all three drugs, a comparable though not normalizing blood pressure decrease was effectuated. Therefore, neither volume factors, nor relative hyperactivity of the renin - aldosterone system nor β-adrenergic hyperactivity can be considered to be prime movers in normoreninemic hypertension. Moreover, the maintenance of a supranormal blood pressure points to the presence of

mechanisms counteracting a decrease in blood pressure to normal levels. The high renin- and aldosterone levels found after long term chlorthalidone treatment were considered to be expressions of such counteracting mechanisms, as were the even higher renin levels measured after spironolactone. Finally, the weight increase observed during treatment with rather high doses of propranolol might represent volume retention as another mechanism counteracting the blood pressure lowering effect of this drug.

The relevance of these considerations is illustrated by the greater effectiveness of the combination of β-blockade and diuretics in the treatment of essential hypertension reported in the literature.

Dank zij de waardevolle assistentie van Miekie Thissen-Jansen kon de biologische bepaling van de plasma renine aktiviteit worden vervangen door een radioimmunologische bepaling. Vele van de bepalingen welke zij verrichtte, zijn in dit proefschrift vermeld. Zij werd bij het verwerven van de bloedmonsters vakkundig geassisteeerd door Angeline van Geel. De in dit proefschrift vermelde aldosteron bepalingen werden verricht door Hans Hofman, Oda Voesten, Frans van Rosmalen en Dick Loozekoot.

Veel dank ben ik verschuldigd aan de studenten en medewerkers van deze universiteit die ten behoeve van het verzamelen van 'normale' waarden bereid waren aan niet altijd even plezierige proefopstellingen mee te werken.

Respekt heb ik voor de patienten die gedurende meer dan een jaar om de 14 dagen de polikliniek bezochten en zo een evaluatie van de behandeling met verschillende antihypertensiva mogelijk maakten. De gewaardeerde medewerking van collega Jan Festen heeft veel bijgedragen tot de voltooiing van dit onderzoek.

Ook dank ik de verpleegkundigen van de polikliniek van de klinische afdelingen die steeds bereid zijn geweest mij bij mijn onderzoek behulpzaam te zijn.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 31 januari 1946 te Amsterdam geboren. Van 1958 tot 1963 bezocht hij het Sint Joriscollege te Eindhoven (H.B.S. B). In 1963 begon hij zijn studie in de Geneeskunde aan de Katholieke Universiteit te Nijmegen. In 1966 behaalde hij het kandidaatsexamen en in 1969 het doctoraal examen. In 1971 legde hij het artsexamen af. Sindsdien volgt hij de opleiding tot internist in de Universiteitskliniek voor Inwendige Ziekten te Nijmegen (directeur Prof.Dr. C.L.M. Majoor).

STELLINGEN

1

De betekenis van de minder dan normale stimuleerbaarheid van de plasma renine aktiviteit bij hypertensie is nog steeds omstreden, mede omdat aan de standaardizering van de meting van de plasma renine aktiviteit en aan de omstandigheden waaronder bloed voor deze meting wordt verzameld onvoldoende aandacht is besteed

Dit proefschrift

2

Indien bij een patient een te lage plasma renine aktiviteit en een lage aldosteron secretiesnelheid gemeten worden, dient overmatig gebruik van drop uitgesloten te worden alvorens te denken aan een overproduktie van een ander - bekend of onbekend - mineralocorticoid

3

Gezien de recente literatuur gegevens betreffende het effekt van het (Des-Asp^1) heptapeptide op de aldosteronproduktie door de zona glomerulosa in vitro, lijkt een vergelijkende studie van het effekt van angiotensine II en van dit biologische derivaat van angiotensine II op de secretiesnelheid van aldosteron bij de mens zinvol

P Fredlund, S Saltman and K J Catt
J Clin Endocrinol Metab 40 746 - 749, 1975

4

In studies betreffende de relatie tussen de aanwezigheid van receptoren voor oestradiol in carcinomateus mammaweefsel en het optreden van klinische remissies door een aantal endocriene maatregelen, dient vermeld te worden of de onderzochte patienten pre- dan wel post-menopauzaal zijn

5

Het is onvoldoende bekend dat tijdens langdurige behandeling met 5-fluorouracil een aanzienlijke stijging van het thyroxinegehalte in het plasma optreedt zonder enig klinisch kenmerk van hyperthyreoïdie.

L. Beex, A. Ross, A. Smals and P. Kloppenborg
Nog niet gepubliceerde waarnemingen.

6

Het ribbeboog - bekkenkam - syndroom kan onder andere bij bejaarde patiënten een simpele verklaring zijn voor in de buik of flank gelokaliseerde pijnklachten.

J. Michels
Ongepubliceerde waarnemingen.

7

Het is in het algemeen aan te raden om bij de statistische bewerking van klinische en biochemische gegevens betreffende patiënten non - parametrische toetsen te gebruiken.

8

Een zorgvuldig opgezette prospectieve klinische studie lijkt noodzakelijk, teneinde de door Kaplan opgesomde argumenten tegen strenge zoutbeperking bij de preventie en de behandeling van preeclampsie te aanvaarden of te weerleggen.

N. M. Kaplan, Clinical Hypertension
Medcom press 1973, New York pp 313 - 314.

9

Voortschrijdend gebruik van de computer bij de opslag van gegevens uit wetenschappelijke literatuur zal leiden tot standaardizering en dientengevolge tot verarming van taalgebruik, waardoor belangrijke nuances verloren kunnen gaan.

Bij het ontwerpen van personenwagens dient voor het aanbrengen van wettelijk verplichte of individueel gewenste vignetten ruimte op het koetswerk gecreeerd te worden, teneinde het rijden niet nog uitzicht-lozer te maken.

26 juni 1975

J. I. M. Drayer